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Burrow Collapse as a Potential Stressor on the Gopher Tortoise (*Gopherus polyphemus*)

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Final report

Abstract: The gopher tortoise is a species of concern throughout the Southeast, and is a keystone species throughout its range. It is federally listed as "Threatened" in part of its range and has been proposed for listing elsewhere. During forest thinning and harvesting, and during military training exercises, tortoise burrows are often accidentally run over. No formal studies of the potential for tortoise injury resulting from burrow collapse had been conducted in the natural environment, however. This research was designed to determine the potential for tortoise injury from the direct crushing of the burrows or loss of life through inability to escape from a collapsed burrow. Forty intentional burrow collapses by heavy equipment were studied. Data were acquired on pre- and post-collapse movement patterns and several general health and physiological measures. No tortoises appeared to have any life-threatening injury, and tortoises normally self-excavated, with excavation intervals ranging from an hour to 85 days. All tortoises remained within a normal home range of their collapsed burrow, resulting in little change in movement patterns after burrow collapse. Changes in home range, number of burrows used, daily movement patterns, or the mean distance moved by the tortoises do not appear significant.

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1 Introduction

Background

The gopher tortoise (*Gopherus polyphemus*) is found only in the south-eastern United States. The species has an existing range from South Carolina to Florida and from the Atlantic coast west to Louisiana (Ernst et al. 1994). It is listed as a federally threatened species in the western part of its range, west of the Mobile and Tombigbee rivers, and a species of concern in the eastern part of its range (U.S. Fish and Wildlife Service [USFWS] 1987). Gopher tortoise populations are shrinking (Auffenberg and Franz 1982), and two of the most cited reasons for this decline is the loss of its habitat, the longleaf pine ecosystem (Noss 1988), and land use. The species has declined by over 80 percent in the suitable remaining habitat (Hermann et al. 2002). About 80 percent of the habitat in the listed range has been lost due to urbanization and agriculture (USFWS 1990) with increased urbanization, highway construction, and poor forestry practice (largely that of unwarranted fire suppression). The remaining suitable habitat continues to be threatened by these factors. A January 2006 petition requested that the USFWS list the tortoise as Threatened in the remainder of its range (Save Our Big Scrub 2006).

In addition to these pressures, there is concern that the species may possibly be declining due to disease. The disease suspected of impacting gopher tortoise populations is Upper Respiratory Tract Disease (URTD), caused mainly by the bacteria *Mycoplasma agassizii* (Brown et al. 1999). Symptoms include an increase in inflammatory cells in the respiratory tract, labored breathing, nasal exudate, conjunctivitis, and lethargy, which can cause dehydration, emaciation, and possibly death. The pressures associated with urbanization may decrease the tortoises' immune system, which may make them more susceptible to URTD.

Two other factors that may be contributing to species decline are a long maturation period and hatchling mortality. Tortoises live approximately 40–60 years in the wild. Because they take approximately 10–20 years to reach sexual maturity (Diemer and Moore 1994; Aresco and Guyer 1999a), the viability and stability of tortoise populations rely on a healthy population of adult tortoises. Also important in the maintenance of tortoise population size is survival of hatchlings to sub-adult and adult life stages, since tortoises mature late and produce few young throughout their life (Seigel

and Dodd 2000; Alford 1980). Hatchlings are at a very high risk of predation with mortality rates estimated in excess of 90 percent (Butler and Sowell 1996; Witz et al. 1992). A small increase in adult mortality may cause demographic instability and a decline in populations.

Gopher tortoises are considered a keystone species and a specialist in their environment (Guyer and Bailey 1993). Tortoises dig burrows that may extend 5 meters down from the surface and 15 meters in length (Diemer 1986; Hansen 1963). These burrows provide shelter and refuge to more than 330 species (Jackson and Milstrey 1989) and, while digging these burrows, they change the composition of the forest by bringing large amounts of soil to the surface. This excess soil is then distributed around the opening of the burrow, allowing for higher plant species richness (Kaczor and Hartnett 1990). The tortoises' role in creation of these species' refugia and increasing species diversity caused ecologists to give the gopher tortoise its keystone status.

Because of the importance of gopher tortoises and their burrows, land use practices such as silviculture, construction, and activities associated with military training are potentially problematic when they occur in gopher tortoise habitat. The vehicles used in these activities can accidentally run over the burrows and possibly collapse them, potentially causing physical harm while entombing the tortoise. Even if these collapse events do not cause injury, they may act as a stressor. This potential increase in stress caused by burrow collapse events may increase the tortoises' susceptibility to disease (e.g., URTD). An event like a burrow collapse may also force tortoises to abandon their burrows, alter their home range or movement, and interrupt their breeding cycle. If tortoise populations are shrinking as a direct or indirect effect of the stress caused by having their burrows collapsed, current military and forestry land management policies will have to be assessed (Seigel and Dodd 2000).

Physiological consequences of environmental stressors

A stressor could be defined as anything that disrupts the normal resting state of an animal, indicated by a change in homeostasis (internal stability). This disruption may cause an increase in circulating glucocorticoids, which are steroid hormones secreted by the adrenal gland in response to an external stressor, such as environmental perturbations that increase energy demands and/or decrease food availability (Wingfield 1994). The hormones promote gluconeogenesis (glucose formation) from non-carbohydrate sources such as skeletal muscle (Harvey et al. 1984).

Pickering and Pottinger (1989) have shown in some species of salmonid fish that the increase in a stressor is directly proportional to the levels of glucocorticoids released. Barton and Iwama (1991) have also documented this phenomenon in several additional species of fish. Initial release of these glucocorticoids is beneficial in that they help the individual mobilize stored energy, increase delivery of nutrients to muscle, and increase immune activity (Sapolsky et al. 2000). When the stressor becomes severe or chronic, however, it can act to override the negative feedback mechanism that is designed to stop the production and release of corticosteroids. When the feedback mechanism is overridden, it causes an increase in glucocorticoids in the bloodstream (Feek et al. 1983). This increase in corticosterone can have detrimental effects such as an alteration of the reproductive cycle and immunosuppression (Bateman et al. 1989).

Prolonged stress can inhibit reproductive function by inducing changes in the pituitary gland and/or in gonadal responses (Greenberg and Wingfield 1987; Rivier and Rivest 1991). In fact, various components of the stress response are able to inhibit all levels of the reproductive axis (Rabin et al. 1988). Male turtles that were subjected to the acute stress of capture and repeated blood sampling showed not only an increase in corticosterone (a glucocorticoid), but also showed a significant decline in plasma testosterone concentrations (Mendonça and Licht 1986; Licht et al. 1985). In addition, female snapping turtles subjected to capture and repeated blood sampling demonstrated various changes in plasma concentrations of sex steroid levels, depending on their reproductive condition (Mahmoud et al. 1989). These studies indicate that a connection exists between increased stress levels (as indicated by elevated corticosterone) and changes in sex steroid hormones.

Burrow collapse

Auffenberg and Franz (1982) thought that tortoises would become entombed and die after their burrows were collapsed. Even if burrow collapse and the resulting entombment period do not prove lethal, this condition may act as a stressor on gopher tortoises. Thus, burrow collapse may cause changes in physiology, health, and behavior. The effects could possibly be seen in abnormal hormonal profiles, altered movement patterns and home range size, a higher rate of burrow abandonment, and/or decreased fecundity (ability to produce offspring). An increase in circulating corticosterone resulting from the presence of a stressor can potentially have a negative impact on general health and immunocompetence.

In other studies on the consequences of burrow collapse, tortoises were only observed to see if they were able to self-excavate after site preparation (roller chopping), but no short (intra-year) or long (inter-year) term monitoring was done to look at the physiological or behavioral consequence. Landers and Buckner (1981) observed 18 active tortoise burrows after site preparation in the fall and had 11 tortoises self-excavate within 3 weeks. The remaining seven burrows were manually excavated and were found to be uninhabited. Diemer and Moler (1982) monitored three tortoises until they self-excavated. All three did so within 8 weeks. In the last known experimental study conducted to date, burrows were collapsed by small tractors as part of a pipeline rights-of-way study (Wester 2004). Almost all the adult and sub-adult tortoises managed to self-excavate (the longest period of entombment was 107 days); however, one tortoise failed to excavate and, after manual excavation, was found dead in its burrow. The cause of death was not known.

Very few studies have explored collapsing of gopher tortoise burrows; the study reported here was also unique in that it was the first one to examine the physiological responses of gopher tortoises to entombment after collapsing of their burrows. It was then possible to follow these animals long term to investigate the physiological and behavioral effects 1 and 2 years after burrow collapse. This information may be vital in helping to learn more about gopher tortoises' reactions to environmental stressors, which in turn may shed light on efforts to save a declining keystone species.

Objectives

Short term (intra-year)

- Assess the extent to which tortoises are physically injured or killed when their burrows are collapsed
- Assess how long entombed tortoises take to self-excavate
- Assess the factors affecting time to self-excavation
- Assess the short-term physiological (stress responsiveness) and behavioral (movement and home range) effects of entombment on gopher tortoises after the collapse of their burrows

Long term (inter-year)

- Assess the long-term physiological (stress responsiveness) and behavioral (movements, home range, burrow abandonment) effects of en-

tombment on gopher tortoises 1 and 2 years after the collapse of their burrows.

Scope

This study was performed at Fort Benning, which is in southwest Georgia, with a portion extending into southeast Alabama. This military installation is 73,533 ha in size, and the areas that support gopher tortoises are forested predominately by loblolly (*Pinus taeda*) and longleaf pine (*Pinus palustris*) with some hardwoods and is being managed toward a longleaf pine ecosystem (Dilustro et al. 2002). In September 2003 and June 2004, 20 and 22 occupied gopher tortoise burrows were collapsed, respectively. All tortoises used in this study (2003 and 2004) were adults; however, they were all beyond the age that plastral growth rings accurately represent age (as a result of wear) so ages could not be determined.

Approach

With the assistance of U.S. Army Engineer Research and Development Center (ERDC) and Fort Benning staff, sites were selected where relatively high densities of tortoise burrows were found and where, so far as could be determined, little forest management or military heavy equipment had operated for several years. Selected burrows were located and their coordinates determined by global positioning system (GPS). Traps were set to collect the resident animals. The tortoises were then evaluated for general health parameters, and blood samples taken to determine stress hormone levels. Blood samples were also taken from each of the tortoises after collapse and excavation to compare the levels of gluconeogenesis found during the pre-excavation sampling. Observations of several physiological and behavioral parameters were also recorded to better evaluate the effect of the collapse. A total of 42 tortoises (20 in fall 2003 and 22 in spring 2004) had their burrows collapsed with either an Armored Personnel Carrier (APC; 10 in 2003) or a logging skidder (10 in 2003 and 22 in 2004).

Method of technology transfer

The information included in this report is one portion of the materials prepared by ERDC to assist installation natural resources and Threatened and Endangered Species (TES) program managers. The gopher tortoise is the first species in a planned series of studies that treat management of Species at Risk as they relate to military installation management plans. The primary means of communicating the tortoise behavior information

will be through publication in the scientific literature, as well as through the availability of this report. The specific data presented are intended to be used in the preparation of biological assessments and biological opinions related to planned Army actions where the gopher tortoise is present. The data also will be used for preparation of management plans, integrated natural resources management plans (INRMPs), and ecological risk assessments involving training and other land-disturbing activities where the tortoise is present. This report will be made accessible through the World Wide Web at URL http://www.cecer.army.mil.

2 Methods

Study site

Surveys were conducted in training compartments D12 and K20 on the Fort Benning military installation (Figure 1) from 27 May to 17 June 2003 to identify active burrows in locations feasible for performing burrow collapses. On 3 September 2003, 10 of these burrows were collapsed with an APC, and on 6 September 2003, the 10 remaining burrows were collapsed using a logging skidder (Table 1).

In April 2004, surveys were done in training compartments O3, O4, and O5 to identify 22 active burrows. Eleven of the burrows were collapsed on 12 June, while the remaining burrows were collapsed on 19 June. Table 2 shows the number of tortoises whose burrows were collapsed by date, sex, and equipment used to collapse the burrows.
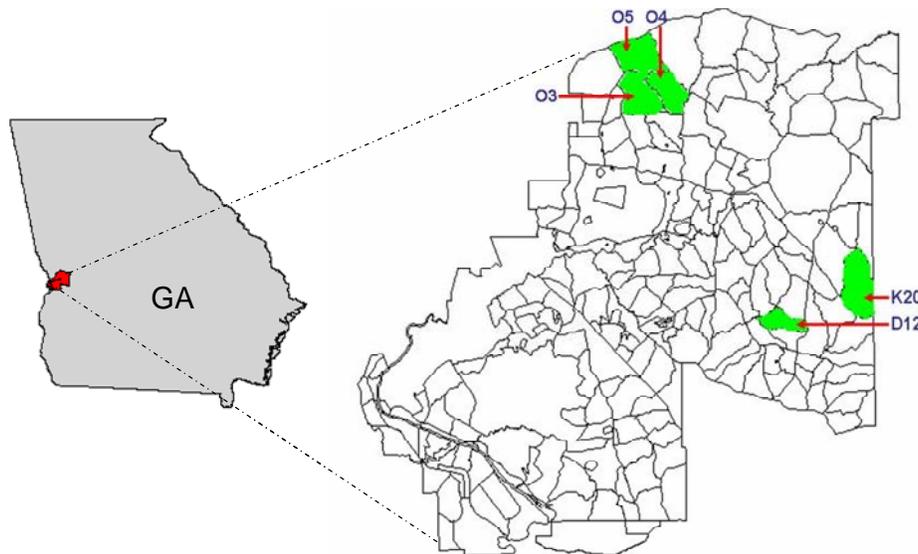
Figure 1. Map showing location of Fort Benning and the training compartments referred to in this report. Compartments where studies were conducted are green.

Table 1. Number of tortoises by site, equipment type, and sex—Fall 2003.

Collapse Equipment	Date	Compartment - D12		Compartment – K20	
		Male	*Female*	*Male*	*Female*
APC	3 Sep	6	4	0	0
Skidder	6 Sep	2	2	3	3
Total		14		6	

Table 2. Number of tortoises by site, equipment type, and sex—Spring 2004.

Collapse Equipment	Date	Compartment - Oscars 03, 04, and 05	
		Male	*Female*
Skidder	June 12	5	6
Skidder	June 19	6	5
Total		22	

Aerial views of the two sites, D12 (14 burrows) and K20 (6 burrows), are shown in Figures 2 and 3, respectively. The D12 compartment is located near the center of the base. The habitat is fairly xeric (dry) and made up mostly of loblolly (*Pinus taeda*) and longleaf pine (*Pinus palustris*) with some upland hardwoods. The soil consists of Troup sandy loam (1 to 10 percent clay). The study site is approximately 31 ha in size. The K20 compartment is at the eastern boundary of the base, and the study site is just over 2 ha in size. The habitat is also quite xeric with an extremely sparse canopy. The community is yellow pine and upland hardwoods with Troup sandy loam soils (1 to 10 percent clay). The 2003 sites (D12 and K20) are approximately 8.2 km apart (Figure 4).

One larger site was studied in 2004 near the junction of training compartments O3, O4, and O5 (Figure 5). Dubbed the "Oscar" site, this area is in the northwest portion of the base and the study site is approximately 138 ha in size, with the habitat being mostly loblolly (*Pinus taeda*) and longleaf pine (*Pinus palustris*) with some hardwoods. Soils at the site consist of Esto (35–60 percent clay), Troup (1–10 percent clay), Troup-Esto and Wagram (2–10 percent clay) sandy loams. This site is 13.1 km from compartment D12 and 32.7 km from compartment K20 (Figure 4). Of the three sites, the Oscar site is much less xeric than the other two (K20 and D12). Streams are within the area reflecting the moister and cooler setting, with more hardwoods and canopy cover. This site is the largest of the three, but the burrows are close enough that the tortoises may be considered one large population.
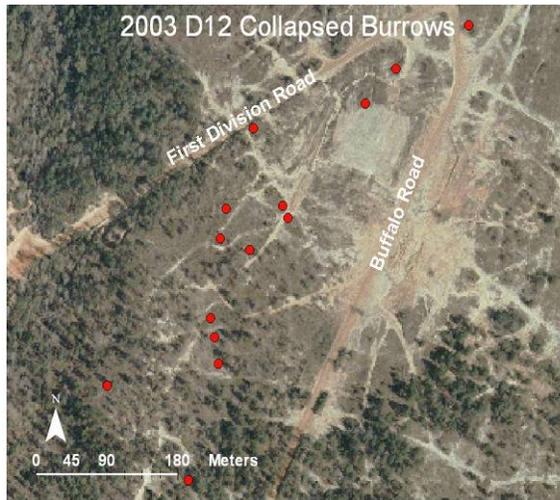
Figure 2. Red dots show locations of burrows that were collapsed in 2003 in compartment D12.
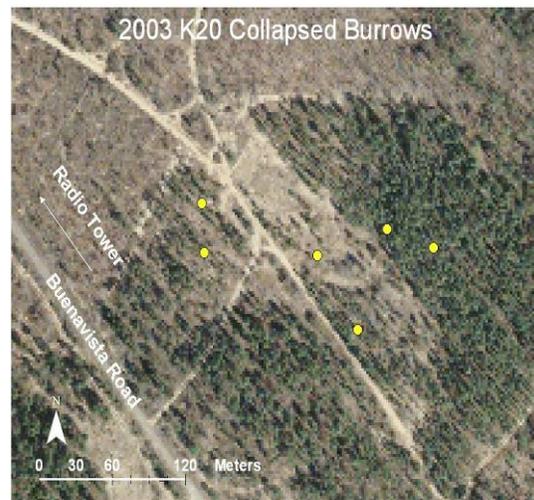
Figure 3. Yellow dots show locations of burrows collapsed in 2003 in compartment K20.
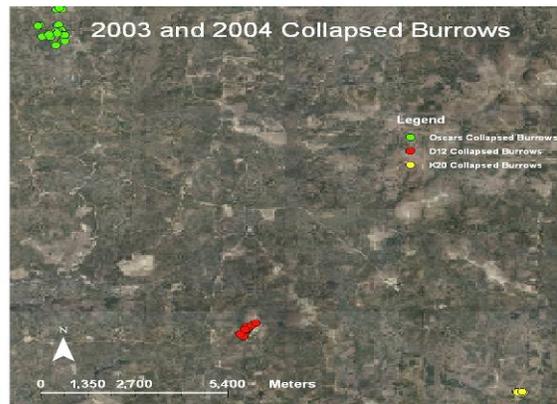
Figure 4. Locations of all three collapse sites for 2003/2004. Green dots are collapsed burrows in 2004, and red and yellow dots are collapsed burrows from 2003.
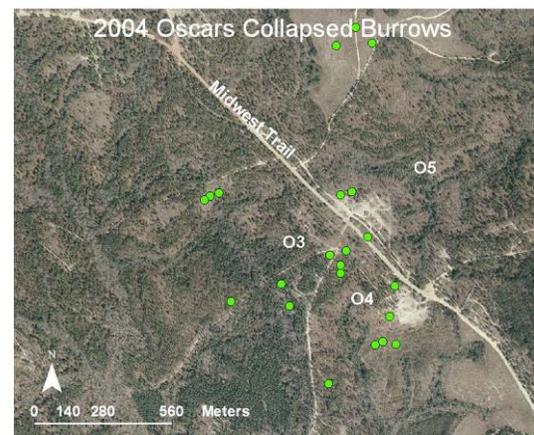
Figure 5. Green dots show locations of collapsed burrows in 2004 in compartments O3, O4, and O5.

Short-term (within the collapse year) monitoring

Active burrows (n = 20 Fall 2003; n = 22 Spring 2004) were located and mapped by GPS at the start of the field season (May 2003 and April 2004). A wire live trap (Tomahawk Live Trap, Tomahawk, WI) was placed at the mouth of each selected, occupied burrow to trap the tortoises. The traps were covered with burlap to make the trap appear to be an extension of the burrow. The burlap also serves to protect captured tortoises from overexposure to the sun. Traps were checked twice daily (9:00 a.m. and 4:00 p.m.) until the tortoise was captured.

After the initial capture, a blood sample was immediately collected to establish baseline corticosterone as outlined in Ott et al. (2000). Each tortoise was then painted with an identifying number on its carapace, perma-

nently marked with a file, and fitted with a radio transmitter (American Wildlife Enterprises, Monticello, FL). Tortoises underwent stress and immune challenges and were returned to the burrow of capture the following day. Tortoises were tracked daily with radio telemetry (Wildlife Materials International, Carbondale, IL) for 30 days before their burrows were collapsed to establish the number of burrows used, the number of times moved, and home range size. After the 30 days of tracking, all tortoises were located by telemetry, re-trapped, and another blood sample was taken.

Just prior to the collapse of the burrows, tortoises were located using telemetry equipment to identify the burrow they were then occupying. These occupied burrows were then collapsed with either an M113 APC (Figure 6) in 2003 or a Timberjack 460D (John Deere, Moline, IL) logging skidder in 2003 and 2004 (Figure 7). The APC weighs approximately 10,900 kg and distributes its weight evenly on tracks, exerting a ground pressure of 8.6 psi. The skidder weighs approximately 15,180 kg and exerts a tire pressure of 6.8 psi. The direction and angle of the approach of the machine was dependent on the layout of the burrow and the surrounding vegetation. The approach of the machine was from the front or back in respect to the mouth and path of the burrow. Burrows were also run over at a perpendicular (across the path), parallel (directly over or above the path), and at a 45-degree angle with regard to the path of the burrow tunnel.

Figure 6. M113 Armored Personnel Carrier.   Figure 7. Timberjack 460D logging skidder.

In 2003 five burrows were collapsed by passing the vehicle across the path (perpendicular) of the burrows, taking one to three passes at each burrow. Four of these collapses were done with a skidder and one with an APC. The remaining 15 burrows were collapsed parallel to (directly over or above)

the path of the burrows; again taking one to three passes at each of the burrows.

In 2004 eight burrows were collapsed parallel to and directly over the burrow path. Of these eight, five were collapsed from the back and three were collapsed from the front with respect to the burrow opening. Six burrows were collapsed perpendicular (across) to the path of the burrow either from the right or left. Eight burrows were collapsed at approximately a 45-degree angle to the mouth and path of the burrow. Of these eight, six were from the back and two were from the front.

The extent of collapse was not quantified in 2003, but a procedure was developed to do so in 2004. To measure collapse extent, a string marked at 1-meter intervals was attached to a 7.6-cm steel washer. A long wood dowel was used to place this measuring device down into the burrow prior to the collapse. The wood dowel was removed, leaving the graduated string and washer in place with a known length of string inside the burrow. Burrows were then collapsed and the string was then gently pulled from the former burrow entrance until resistance was met, enabling us to calculate the length of the burrow that had collapsed by recording the length of string withdrawn, and comparing it with the length remaining inside. Collapsed burrows were monitored twice a day until there was evidence that the entombed tortoise had self-excavated.

Tortoises were tracked daily upon self-excavation from the collapsed burrow. They were re-trapped as soon as possible to collect another blood sample to measure corticosterone levels. Stress and immune challenges were also re-administered at this time. Tortoises were released the following day to their burrow of capture and tracked daily for a further 14 days to establish the number of burrows used, the number of times moved, and home range size. Tortoises were trapped after the 14 days of post-emergence tracking for another blood sample.

Movements

Pre- and post-collapse movement patterns were compared. Telemetry was done using receivers and a six-element Yagi antenna (Wildlife Materials International). Tortoises were tracked daily at midday post-collapse for 14 days, and the number and location of burrows used, number of times moved, and their new home ranges were compared with data obtained from their movements for the 14-day period prior to burrow collapse.

Home ranges were calculated using the minimum convex polygon (MCP) method (Mohr 1947).

Soil

Soil data were obtained for the study sites from Fort Benning's data repository in the form of geographic information system (GIS) Soil Survey Geographic (SSURGO) maps. Five soil samples were collected at each burrow in the Oscars (2004 study site) at 20-cm intervals to a depth of 1 m to profile the collapse zone that the tortoise would be excavating through. All samples were dried, strained through a 2-mm sieve to remove debris, mixed with a dispersing agent, and finally strained through a 53-micron sieve to separate coarse material from fine. This coarse-to-fine ratio was used as a crude indicator of clay content to confirm the accuracy of SSURGO soil maps designation of the specific burrow sites.

Weather

Data for temperature and precipitation for both 2003 and 2004 were obtained from the Columbus, GA, weather station and are available at www.weather.gov.

Physiological tests

To investigate the physiological response to burrow collapse, several factors that indicate body condition and stress responsiveness were used. These parameters were assessed before and after collapse of the burrows to monitor tortoises' response to having their burrows collapsed and being entombed. These measures were then correlated to the amount of time the animals were entombed and also compared to pre-collapse levels. A hematocrit—the proportion of the blood that consists of packed red blood cells, an indicator of hydration status (Peterson 2002)—body condition index (CI), and the residuals of mass to straight carapace length (Reist 1985) were obtained to see if they correlated to how long it took the tortoises to self-excavate.

Stress responsiveness

Corticosterone is a glucocorticoid that is the end result of the hypothalamic-pituitary-adrenal axis, and it is the hormone generally used as an indicator (biomarker) of stress (Sapolsky et al. 2000). In this study, stress responsiveness was measured in two ways: (1) by measuring the amount of circulating corticosterone in the blood and (2) by conducting a challenge

to assess adrenal competence using adrenocorticotropin hormone (ACTH) to assess the maximal levels of corticosterone that can be produced by the tortoises' adrenal glands (Hopkins et al. 1999). Before and after the burrow collapse, a blood sample was collected from each participating tortoise. The blood sample was assayed for circulating corticosterone levels using standard radioimmunoassay techniques as outlined in Mendonça et al. (1996). ACTH was obtained from Sigma Co. (St. Louis, MO) and was from one of two production lots. One lot was used for both the 2003 and 2004 studies, while the other was used in the 2005 study.

To assess each gopher tortoise's ability to mount a maximal corticosterone level, each was injected with ACTH, dose validated by Kahn et al. (2007), pre- and post-collapse. A blood sample was collected 4 hours post-injection and again analyzed for corticosterone using standard radioimmunoassay techniques. Normal, healthy tortoises will show low baseline levels of corticosterone, followed by a high peak in corticosterone 4 hours after the ACTH injection.

A concurrent study addressed the tortoise's immune system (T cell and B cell) responses to collapse of their burrow. That portion of the study will not be addressed in this report.

Long-term (between years) monitoring

Tortoises from the short-term studies (2003 and 2004) were re-captured from D12 and the Oscars in 2005 at the start of the active season (April). In addition to these animals, 15 other tortoises (5 from D12 and 10 from the Oscars) whose burrows had not been collapsed were trapped from the same population to serve as controls. A blood sample was collected to assess hormone levels. Long-term effects were assessed by documenting any changes in body CI and stress responsiveness, which were performed the same way as the previous year. These results were compared with what was found in the short-term study. Burrow use by tortoises was also examined to see if collapsed burrows were used again or abandoned. The number of burrows used and home range size were again calculated using MCP, and these data were compared with what was observed in the short-term part of the study.

Data analysis

All data were tested for normality and homogeneity of variance. Only a few of the analysis of variance (ANOVA) comparing corticosterone values had

to use log-transformed values to correct for heterogeneity of variance. A regression analysis of the effect of number of days to self-excavation on physical factors (i.e., amount of burrow collapse) was done as well as pre- and post-collapse physiological factors (i.e., CI, hematocrit, and baseline corticosterone). Additional regression analyses were used to determine the relationship between baseline corticosterone, extent of collapse, and point of self-excavation. CI was obtained by calculating the residuals of tortoise mass to straight carapace length (Schultze-Hostedde et al. 2004).

Home range of both experimental (burrow collapse) and control tortoises was determined with the minimum convex polygon method. Differences in movement pattern parameters (i.e., number of burrows used, distance moved, number of times moved, home range size) before and after the collapse were analyzed by repeated measures ANOVA. Movement parameters of experimental and control tortoises were statistically compared by ANOVA. ANOVA was also used to determine effects of sex, soil type, and machine type on days until self-excavation. An ANOVA was used to determine if angle or direction of the collapse had an effect on self-excavation times. Soil type was determined in two ways. Burrows from the 2004 study were placed in either a high clay content or a low clay content category on the basis of their location on SSURGO soil maps and an ANOVA used to determine if soil category had an effect on self-excavation time. Additionally, soil samples (five evenly spaced samples to a depth of 1 m/burrow) were obtained for these same burrows and a coarse-to-fine ratio analysis conducted. The average coarse:fine ratio was calculated from the five samples/burrow and these values compared by ANOVA between burrows in the high versus low clay categories.

A repeated measures ANOVA was used to compare response to ACTH challenge within a study season as well as changes in all physiological parameters of experimental tortoises between years. An ANOVA was used to compare physiological parameters between experimental and control tortoises. Significance is defined as having a p value < 0.05.

3   Results

Amount of collapse

Fall 2003

Using a visual assessment, the skidder appeared to effectively collapse the burrows whether running over either the track or the mouth of the burrow. The burrow's tunnel caved in and the mouth became closed off. In comparison, the APC did not seem to effectively collapse the burrows. This was true whether it passed over the track or the mouth of the burrow. Only minimal, if any, damage was done to the burrow tunnel; however, the mouth was fully or partially closed when the track passed directly from front to rear. In some cases, multiple passes were needed to effectively close the burrow mouth.

Spring 2004

The extent of collapse ranged from approximately 0.5–2.25 m from the mouth of the burrow. There was no difference in amount of collapse based on angle or direction of collapse (front vs. back $p = 0.2$; perpendicular vs. parallel $p = 0.7$; parallel vs. angle $p = 0.9$; perpendicular vs. angle $p = 0.7$).

Days to self-excavation

Fall 2003 (3-29 September)

All ten tortoises in the burrows collapsed by the APC excavated themselves in 3 to 13 days, with nine of ten taking less than a week to do so (Figure 8). Two of the ten tortoises continued using their burrows after the collapse, five moved to neighboring burrows, and three of the tortoises dug new burrows nearby. It appeared that none of the ten tortoises was physically harmed in the APC burrow collapse.

In the September 2003 study, it was found that nine of the ten tortoises in burrows collapsed by the skidder excavated themselves. They did so within 2 to 13 days, with approximately half (four of nine) taking 13 or more days to do so (as compared to 5 to 6 days for APC tortoises). All nine tortoises moved to neighboring burrows upon self-excavating. One tortoise whose burrow was collapsed with the skidder was manually excavated on Day 23 due to it becoming late in the season. The tortoise was inspected and ap-

peared healthy. Therefore, none of the ten tortoises appeared to have been physically harmed during the skidder burrow collapse.
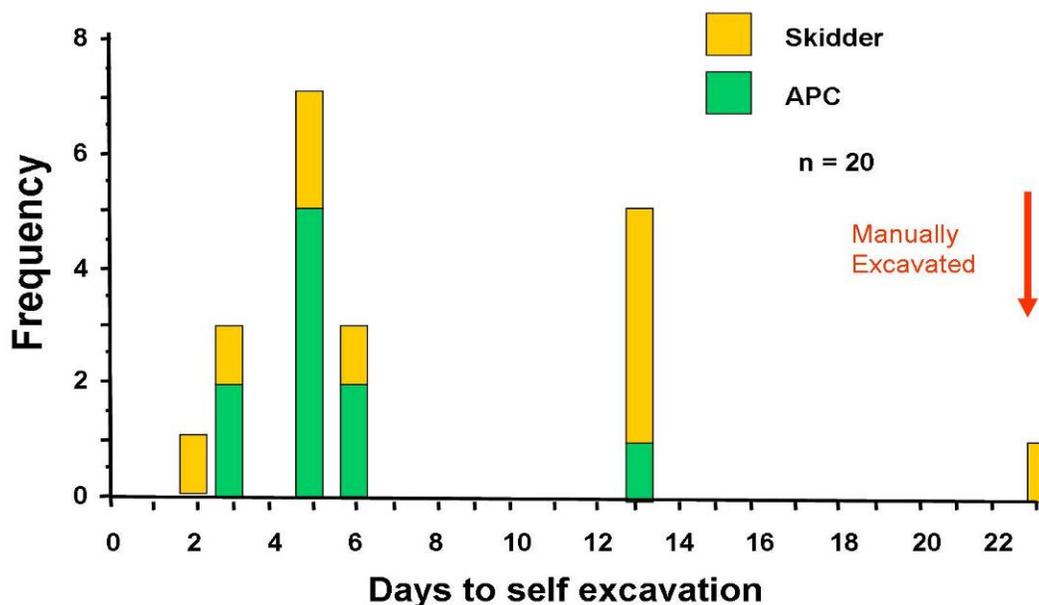
Figure 8. Timeline of tortoise self-excavation in Fall 2003. Red arrow shows tortoise manually excavated.

Summer 2004 (12 June–12 September)

In the first collapse group (12 June 2004), times to self-excavation ranged from just under 2 hours after the collapse to 41 days (Figure 9). The majority (n=6) of the animals had emerged within 7 days of the collapse. The second collapse group (19 June 2004) exhibited a different profile (Figure 9). The first tortoise emerged 3 days after the collapse and most (n=6) emerged within 16 days post-collapse. The next to last tortoise to emerge did so on Day 51. Finally, the last tortoise of this group emerged 85 days after the collapse date (Figure 9). The average time interval to self-excavation in this group was significantly greater (p = 0.018) than those found in the September 2003 group, as well as the group collapsed a week earlier the same year (12 June 2004; Figure 10). As in 2003 (p = 0.3), the difference between the sexes (p = 0.29) was not significant in 2004 for time until self-excavation (Figure 11).
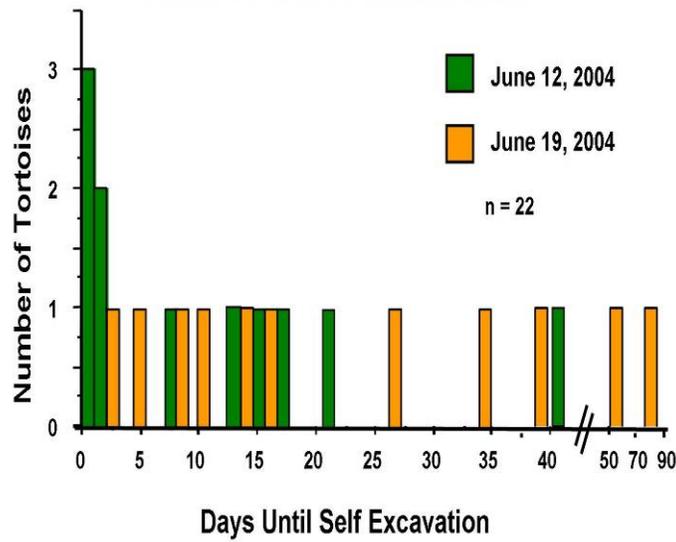
Figure 9. Timeline of tortoise self-excavation—Summer 2004.
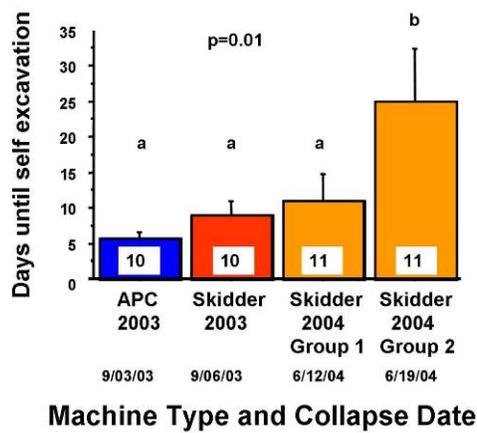
Figure 10. Differences in excavation time subdivided by collapse date and machine type for 2003 and 2004. Tortoises from Group 2 in 2004 took significantly longer (p = 0.01) than the other three collapse dates.
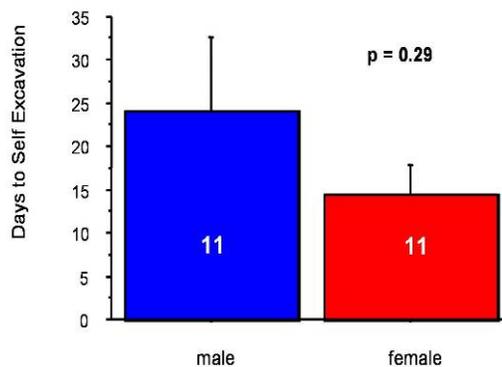
Figure 11. Difference in self-excavation time based on sex—Summer 2004.

Although the extent of collapse was not correlated with time to self-excavation (Figure 12), it was significantly associated with the tortoise excavation point (Figure 13). Tortoises were also significantly ($p = 0.02$) more likely to abandon burrows if they exited farther away from the original mouth (Figure 14).

Of the 22 tortoises that had their burrows collapsed in 2004, 11 (50 percent) stayed at the collapsed burrow, 10 (45.5 percent) moved to an existing burrow not used in the 30-day tracking period prior to the collapse, and 1 (4.5 percent) moved to a previously used burrow.
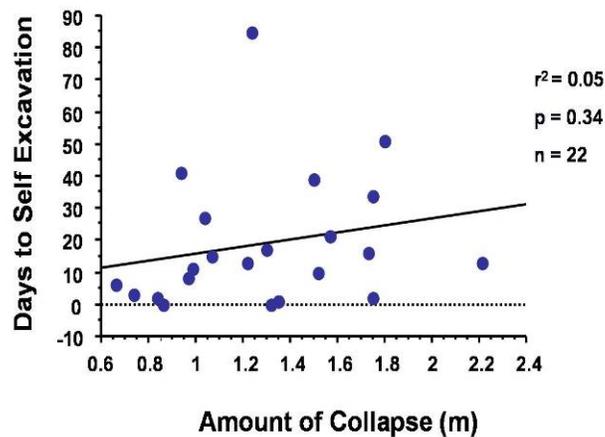
Figure 12. Extent of burrow collapse effect on self-excavation—Summer 2004.
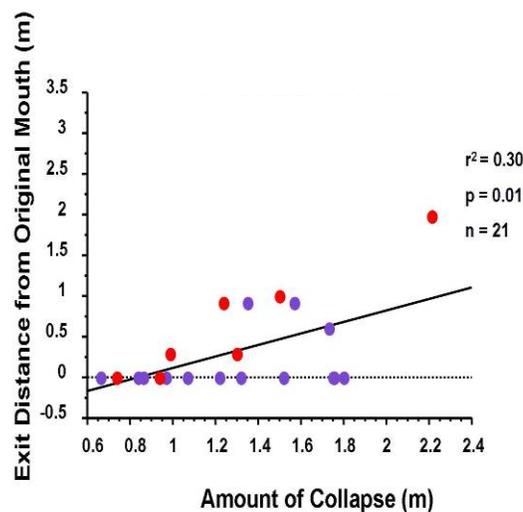
Figure 13. Effect of collapse extent on excavation exit point—Summer 2004.
Red dots represent burrows that were abandoned.
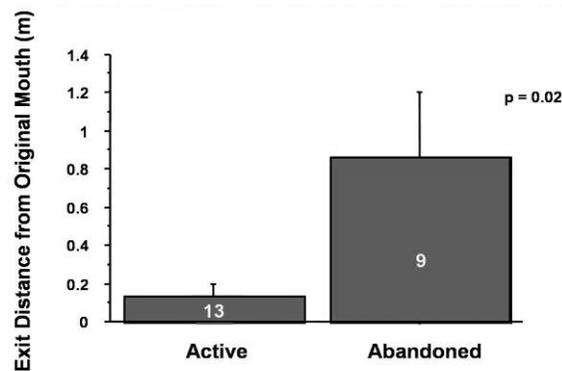
Figure 14. Effect of exit point on abandonment status—Summer 2004.

Movement patterns

2003

The mean number of burrows used and times moved pre-collapse were 3.3 and 3.1 respectively compared to 2.0 (burrows used) and 1.7 (times moved) post-collapse. Difference in the number of burrows used (p = 0.37) or number of times moved (p = 0.78) was not significant for the tortoises when comparing pre-collapse and post-collapse movement behavior. Difference in movement based on sex (p = 0.31) was also not significant. Home range was not calculable due to the small number of burrows used by the tortoises. To calculate and compare home range, the mathematics require that the tortoises must have used a minimum of three burrows both pre- and post-burrow collapse. Since a large number used only one or two burrows the entire period, no calculations were performed.

2004

The mean number of burrows used and times moved pre-collapse were 2.1 and 1.6, respectively, compared to 1.5 (burrows used) and 1.0 (times moved) post-collapse. There was no significant difference in the number of burrows used (p = 0.33), number of times moved (p = 0.13), or mean distance moved (p = 0.15) for the tortoises when comparing pre-collapse and post-collapse movements (Figure 15). Movement was not significantly different based on sex (p = 0.29).
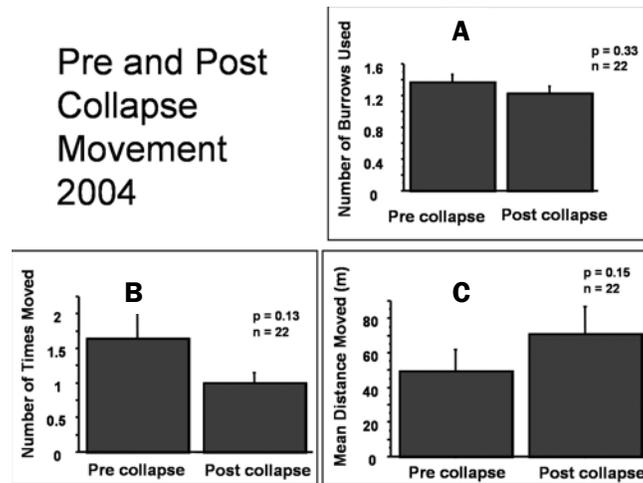
Figure 15. Pre- and post-collapse movement of tortoises in 2004. Movements observed were (A) number of burrows used, (B) number of times moved, and (C) mean distance moved.

2005

When the 2005 movement behavior of the tortoises that were in the 2003 burrow collapse study were compared to that of control tortoises (i.e., whose burrows had not been collapsed), there were no significant differences in the number of burrows used ($p = 0.57$), number of times moved ($p = 0.15$), or home range ($p = 0.81$). A repeated measures ANOVA comparing the movement of the tortoises in 2005 to observed movement in 2003 showed no significant difference in the number of burrows used ($p = 0.7$), the number of times moved ($p = 0.8$), or the mean distance moved ($p = 0.2$).

When the 2005 movement of the tortoises from the 2004 burrow collapse study were compared to that of control tortoises (burrows not collapsed), there were no significant difference in number of burrows used ($p = 0.97$), number of times moved ($p = 0.6$), mean distance moved ($p = 0.6$), or home range size ($p = 0.2$) (Figure 16). A repeated measures ANOVA on movement of the 2004 collapse study tortoises comparing their movements in 2005 and 2004 showed a significant increase in the number of burrows used (3.1 vs. 4.2; $p = 0.03$) and the number of times moved (2.8 vs. 4.9; $p = 0.04$). The difference in the mean distance between moves tended to be higher, but this difference was not significant (84.0 m vs. 143.0 m; $p = 0.06$).
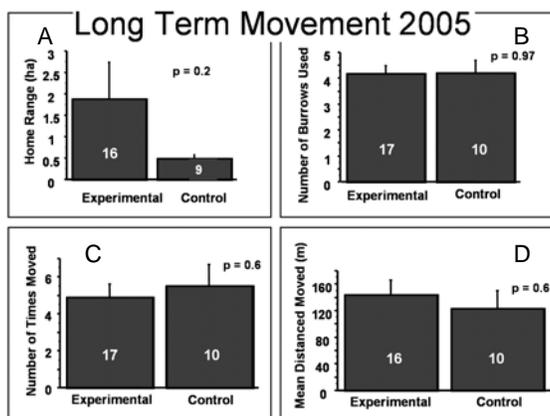
Figure 16. Movements of experimental and control tortoises in 2005, 1 year after burrow collapse. Movements observed were (A) home range, (B) number of burrows used, (C) number of times moved, and (D) mean distance moved.

Weather effects

2003

The weather profiles were similar for the two collapse dates (Figure 17) so it was not considered a variable in time to self-excavation during 2003. Precipitation between the two collapse events was minimal.
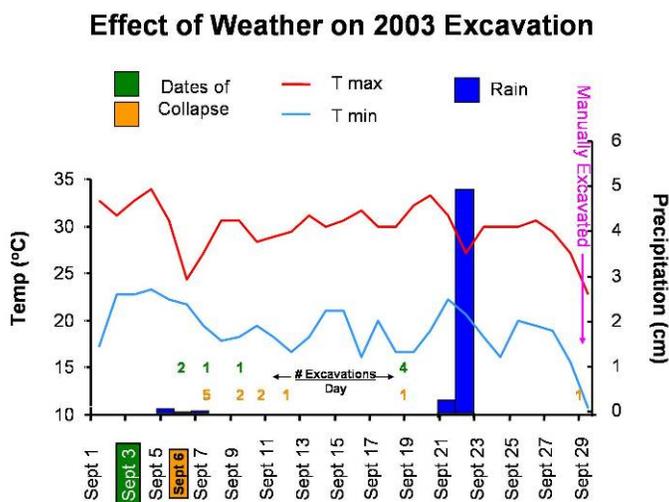
Figure 17. Weather effect on self-excavation time in 2003. Green and yellow numbers on graph represent number of tortoises per day that self-excavated.

2004

While the 7-day temperature profile before the two collapse dates (separated by a week: 12 and 19 June 2004) did not differ considerably, the precipitation pattern the 7 days before the two dates differed considerably

(Figure 18) and may have contributed to the amount of time it took to self-excavate. In the week before the first collapse date (6–12 June), approximately 0.9 cm of precipitation fell on the site, which was roughly 6.4 percent of the total rainfall for the month of June. In the week before the second collapse date (13–19 June), however, 9.9 cm of precipitation fell on the site, more than ten times as much as that of the preceding week, constituting 69.7 percent of the total rainfall for the month.
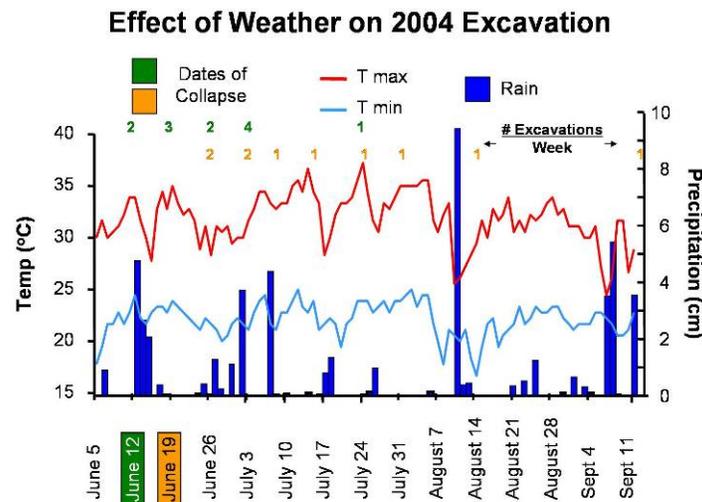
Figure 18. Weather effect on self-excavation time in 2004. Green and yellow numbers on the graph represent number of tortoises per week that self-excavated.

Soil effect

2003

Based on the GIS SSURGO soil maps available for Fort Benning, all of the collapsed burrows in compartments D12 and K20 were in Troup sandy loam. Troup soil types are classified as having between 1–10 percent clay, so these burrows were classified as low clay content burrows.

2004

Based on the GIS SSURGO soil maps available for Fort Benning, all of the collapsed burrows in the Oscars area were in Troup loamy sand, Wagram loamy sand, Troup and Esto loamy sand, or Esto sandy loam. Troup and Wagram soil types are classified as having 1–10 percent clay and these burrows were classed as low clay content burrows. Esto soil type is classified as having 35–60 percent clay and these burrows were classed as high clay content burrows. Of the five burrows where it took tortoises longer than 30 days to self-excavate, four of them were in high clay content soils

(Esto) (Figure 19). When burrows were divided into two categories of high vs. low clay content, based on the GIS SSURGO soil maps, the tortoises from burrows in the high clay content category had significantly longer times until self-excavation (Figure 20).
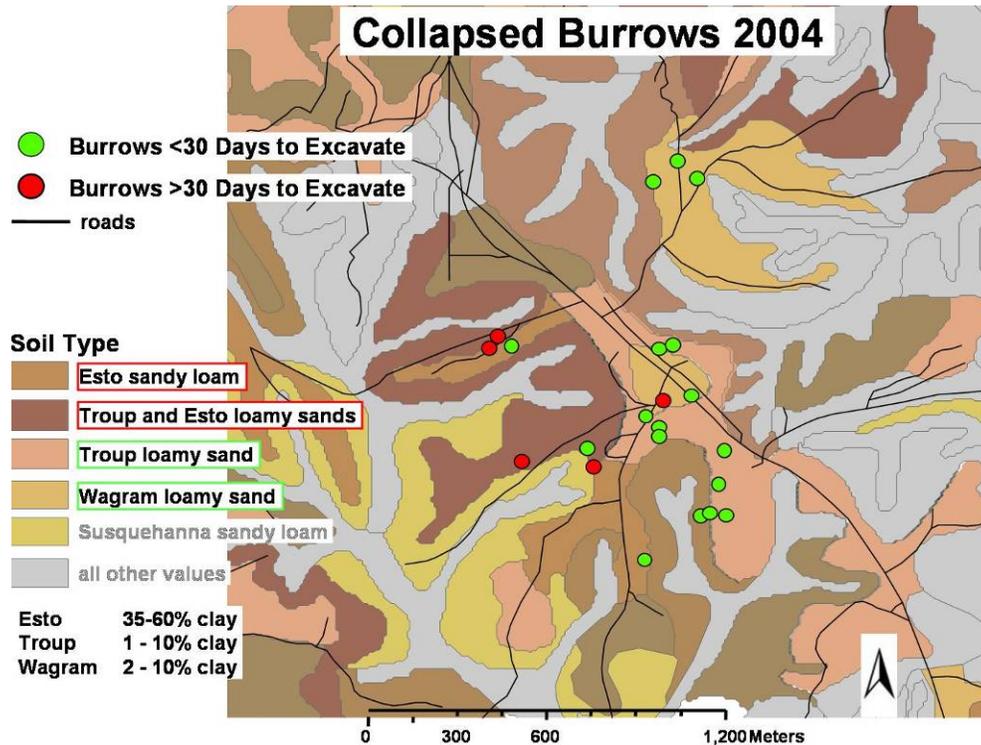
Figure 19. Soil map from 2004 showing the location of the collapsed burrows. High clay content soils are represented by the two darker colors. Red dots indicate burrows where tortoises took in excess of 30 days to self-excavate.
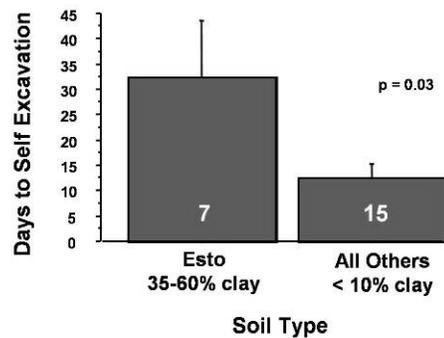
Figure 20. Effect of soil clay content on self-excavation rate in Summer 2004.

To crudely validate the SSURGO soil maps, a fine-to-coarse ratio was performed on all burrows in the Oscars area as this site had more than one soil (clay %) classification. The percent of fine material in each of the five samples per burrow were averaged and these averages placed into high and low clay groups based on the SSURGO soil maps. An ANOVA showed a significant (p=0.0004) difference in the % fine material between the high (n=7) and low (n=15) clay content burrows, confirming that burrows categorized as high in clay content were, in fact, significantly higher in % fine composition.

Short-term physiological responses

Condition index and hematocrit

No significant relationship was found between post-collapse hematocrit or pre-collapse CI and the number of days until self-excavation in 2003 or 2004 (Figure 21 A and B, respectively).
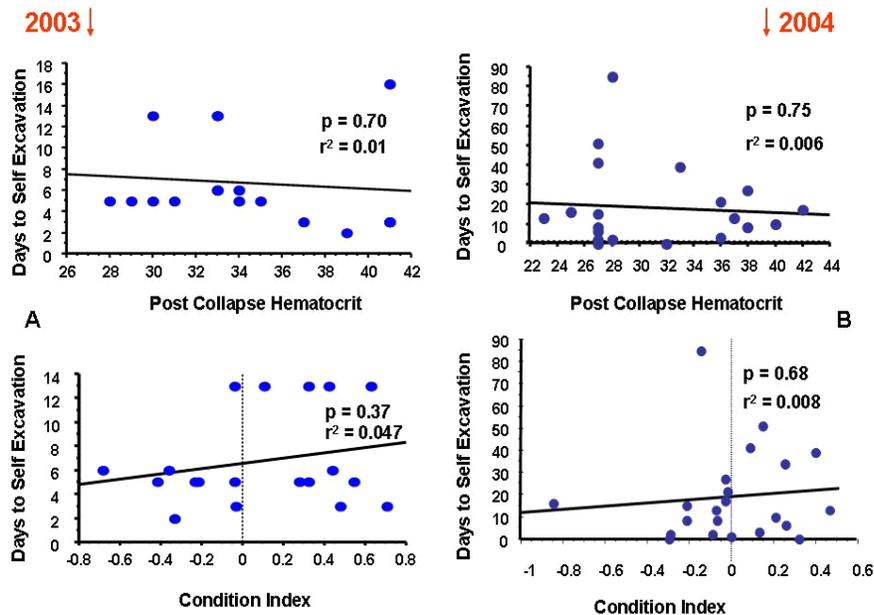
Figure 21. The effects of pre-collapse CI and post-collapse hematocrit on time to self-excavation of tortoises in (A) 2003 and (B) 2004.

Baseline corticosterone

The baseline corticosterone levels of tortoises sampled approximately 30 days before their burrows were collapsed did not show a significant relationship to the amount of time the tortoises remained entombed in the Fall 2003 and Summer 2004 experiments (p=0.93 and p=0.89, respec-

tively). Thus, tortoises with high corticosterone levels before collapse did not tend to remain entombed longer.

Baseline corticosterone levels after tortoises self-excavated were, however, related to entombment interval. In the first burrow collapse study (Fall 2003), baseline corticosterone levels on the first day the animals were captured after they self-excavated from a collapsed burrow were significantly, positively correlated (r^2=0.25; p=0.04) with the number of days the tortoises were entombed (Figure 22). However, most (75 percent) of the variation in baseline corticosterone was not explained by length of entombment. Additionally, almost all (i.e., 15 of 17) of the baseline corticosterone levels were below 12 ng/mL, the value considered to be in the high normal range in this population (Kahn et al., submitted). The one tortoise that was manually excavated after 23 days of entombment had a baseline corticosterone level of 10 ng/mL. Inclusion of this value in the regression analysis minimally alters the overall pattern (i.e., r^2=0.22, p=0.044).
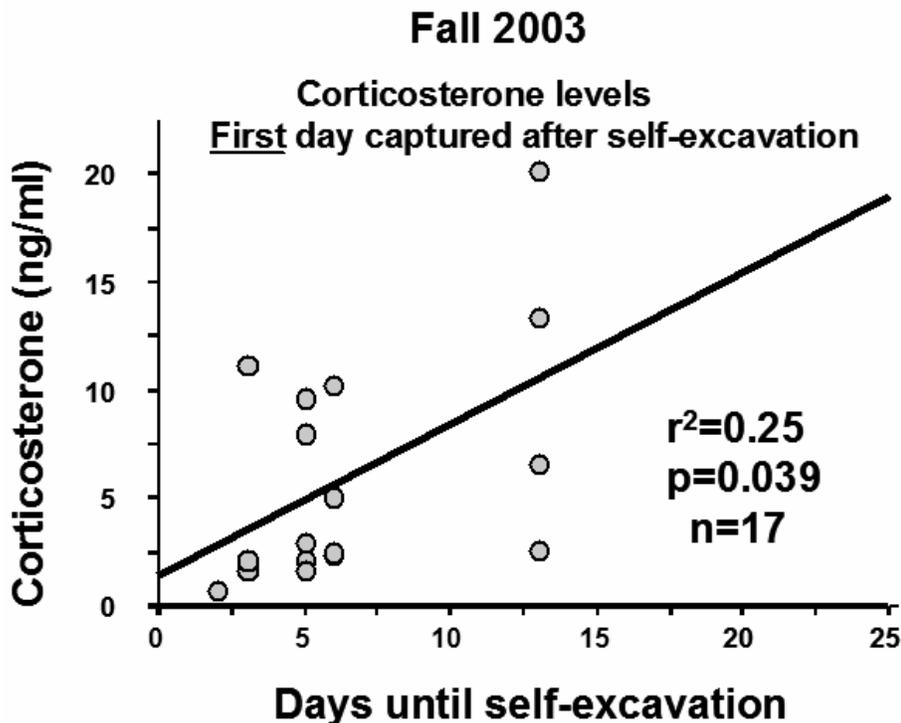
Figure 22. Baseline corticosterone levels of tortoises the first day they were captured after self-excavation in relation to number of days entombed (manually excavated tortoise not included in analysis), Fall 2003.

The number of days between the date of self-excavation and the date when the first blood sample was taken after self-excavation varied among tortoises (range: 1-7 days) because not every tortoise could be trapped in the same time interval after excavation. However, a step-wise regression analysis found that days to self-excavation (p=0.045), not days between self-excavation and blood sampling (p=0.63), was the variable that significantly correlated with baseline corticosterone levels.

In the 2004 experiment, when many entombment times were significantly greater than in 2003 (see previous section), the positive relationship between baseline corticosterone levels and days to self-excavation was even stronger (r^2=0.45, p=0.001; Figure 23). Half of the values were above 12 ng/mL, with the highest value of 40 ng/mL found in the tortoise that was entombed for 85 days. Again, there was considerable variation in the length of time it took to capture animals after they self-excavated (i.e., 0−39 days). A multiple regression analysis indicated that (1) days to excavation significantly (p=0.0005) described 48 percent of the variation in post-excavation corticosterone level and (2) the length of time between self-excavation date and capture (first post-excavation blood sampling date) also contributed significantly (p=0.047) to the relationship. The parameter that explained most of the variation in baseline corticosterone level at the first capture after self-excavation was the total number of days between collapse and sampling (days from collapse to capture) (r^2=0.56; p=0.0001).

In the 2003 experiment, males seemed more affected by the amount of time entombed than females: male baseline corticosterone levels were significantly correlated with entombment period (p=0.02) while those of females were not (p=0.54, Figure 24). A similar sexually dimorphic pattern in baseline corticosterone levels in relation to entombment time was observed in males (p=0.02) and females (p=0.26) whose burrows were collapsed in Summer 2004 (Figure 25).
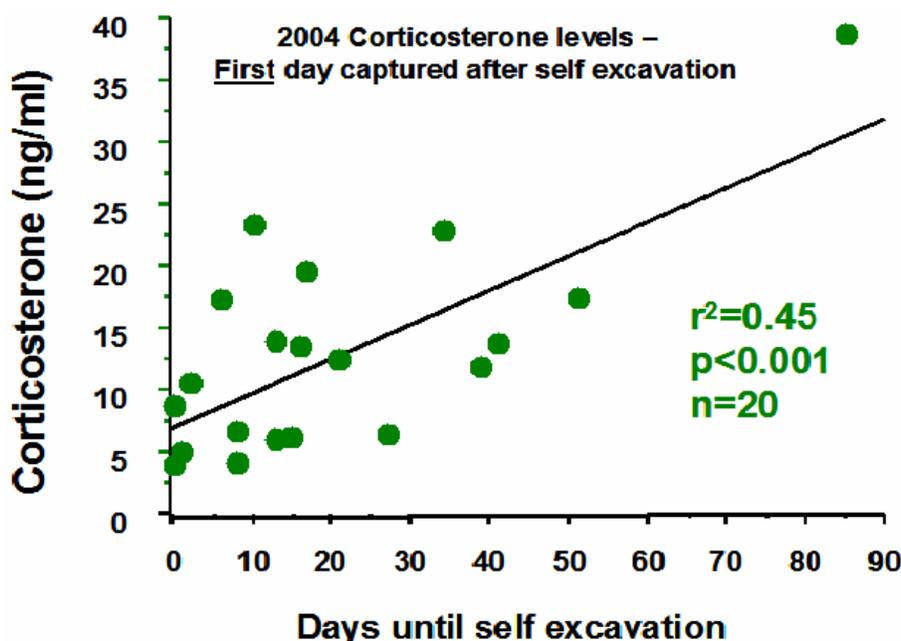
Figure 23. Baseline corticosterone levels on the first day tortoises were captured after they self-excavated in relation to number of days they were entombed, Summer 2004.
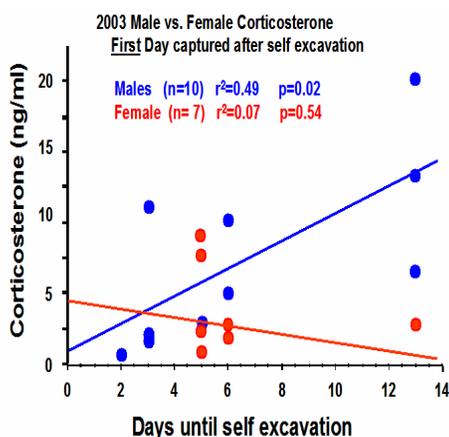
Figure 24. Baseline corticosterone by sex in relation to entombment time, Fall 2003.
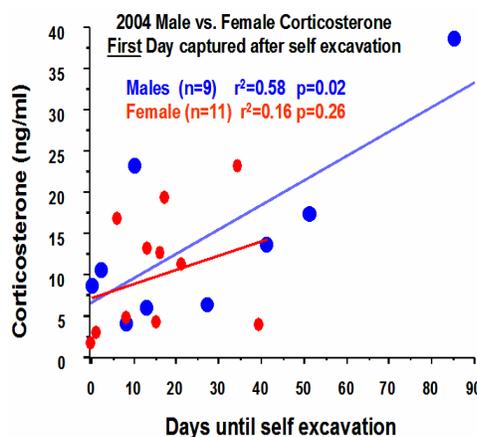
Figure 25. Baseline corticosterone levels by sex in relation to entombment time, Summer 2004.

When tortoises were recaptured and re-sampled approximately 10–14 days after the date they were first trapped after excavation, the relationship between baseline corticosterone and entombment changed between the sexes. In this sample, female baseline corticosterone was now significantly correlated with entombment period, while that of males was not (Figure 26), suggesting females may experience a delayed corticosterone response to entombment while males can recover (at least in terms of baseline corticosterone levels) more quickly from entombment. This finding should be treated with caution, however, because sample sizes are

small and only two animals (one of which was the tortoise manually excavated) drive the significant relationship between Day 10 baseline corticosterone and entombment interval found in females. A repeated measure ANOVA found no significant change in corticosterone between the two sample periods (i.e., the first capture after excavation and Day 10) in either sex (although, again, time until self-excavation was a significant covariate). Unfortunately, a Day 10 sample was not taken during the 2004 experiment to determine if this same pattern would be observed.
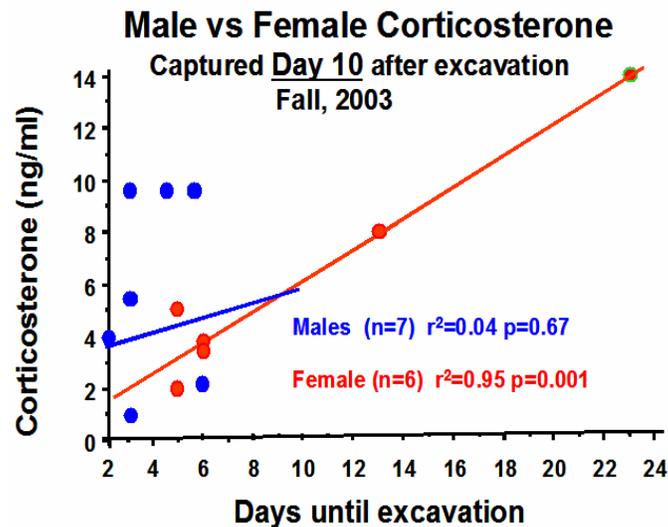
Figure 26. Baseline corticosterone levels of males and female tortoises captured approximately 10 days after the post-excavation sample, Fall 2003.

No significant difference existed was found between years or between sexes in baseline corticosterone levels of tortoises captured and sampled before their burrows were collapsed (Figure 27). However, difference was significant (p=0.0002) between years (but not sex) in the baseline corticosterone levels when animals were first captured and sampled after they had self-excavated from their collapsed burrow: tortoises in the 2004 study had significantly higher corticosterone levels than 2003 tortoises at this sample (Figure 28). When time to self-excavation is included in the analysis as a covariate, however, the difference in post-excavation corticosterone levels between years was not significant (p=0.42).
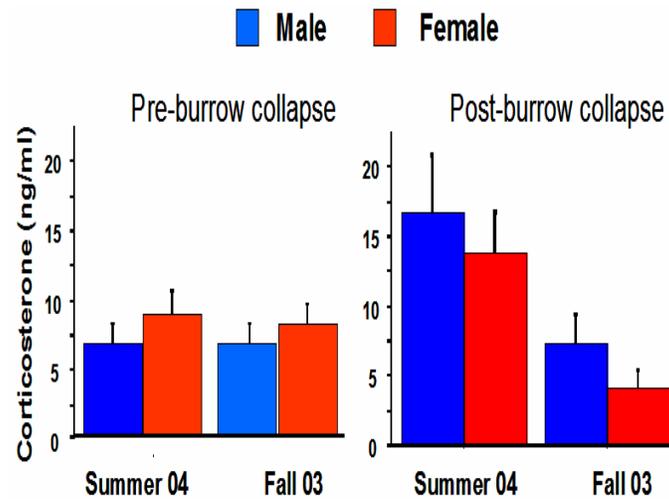
Figure 27. Baseline corticosterone levels before and after burrows collapse by year and sex.

ACTH challenge

2003

During the 2003 experiment, tortoises received either an ACTH or a control saline injection at 30 days (N=8, 8) and 7 days (N=7, 5) before burrow collapse and on the first day captured after self-excavation (N=7, 11). In each of these challenges, the saline injection treatment did not significantly increase circulating corticosterone levels. Thus, the experimental manipulation (i.e., injection and temporary transport and holding of the animals) did not itself appear to elicit a stress response.

In contrast, tortoises that received an ACTH injection exhibited significantly elevated corticosterone levels 4 hours after injection when tested approximately 30 and 7 days before being subjected to burrow collapse (p=0.02 and p=0.003, respectively; Figure 28a, b). However, when tortoises were administered ACTH injections on the first day they were captured after self-excavation, they did not exhibit an increase in corticosterone levels (p=0.11; Figure 28c).
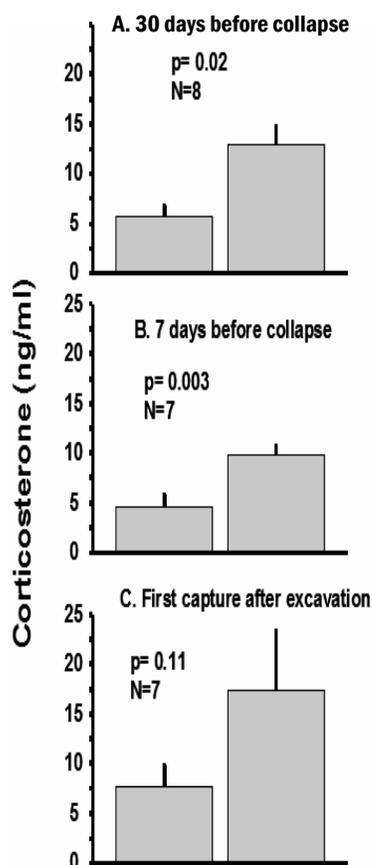
Figure 28. Corticosterone levels of tortoises in Fall 2003 upon capture and 4 hours after being given an ACTH injection (a) 30 days before their burrows were collapsed, (b) 7 days before their burrows were collapsed, and (c) on the first day they were captured after they excavated. Saline injection controls all had corticosterone levels lower than the initial levels 4 hours after injection and are not shown.

2004

Because there was no difference in response to the ACTH challenge between the two tests done before the burrow collapse in 2003, only one ACTH challenge was done 30 days before burrow collapse in 2004. Additionally, since results of the 2003 saline controls indicated that the challenge administration and handling did not cause an increase in corticosterone, only two animals were injected with saline in the 2004 burrow collapse (allowing an increase in sample size for the ACTH group). As in 2003 the corticosterone levels of these two saline controls were not different than their initial levels when they were sampled 4 hours after injection. This result indicates again that the experimental manipulation (i.e., injection, transport, and temporary holding) does not itself increase corticosterone levels.
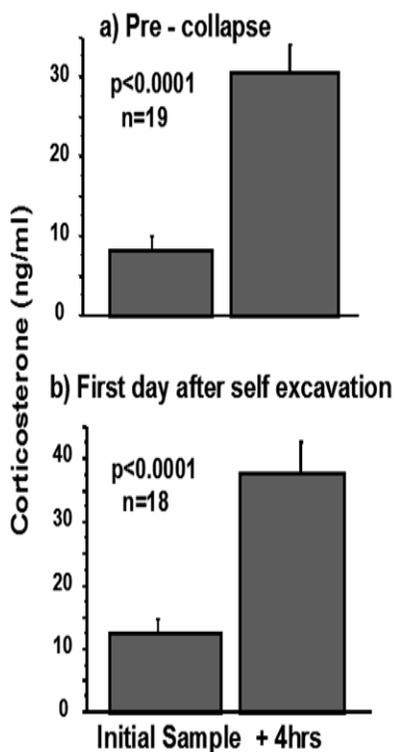
Figure 29. Corticosterone levels of tortoises upon capture and 4 hours after being given an ACTH injection (a) 30 days before their burrows were collapsed, and (b) on the first day they were captured after they excavated, Summer 2004.

As in the previous experiment, tortoises given ACTH approximately 30 days before their burrows were collapsed again exhibited significantly elevated (p=0.0001; N=19) corticosterone levels when sampled 4 hours after injection (Figure 29a). However, unlike the Fall 2003 experiment, tortoises given ACTH on the first day they were recaptured after self-excavation also responded significantly (p=0.0001, N=18) to the challenge (Figure 29b).

In Summer 2004, the mean plasma corticosterone levels 4 hours after administering the ACTH challenge were significantly higher than those observed during the Fall 2003 experiment, both before burrow collapse (e.g., 27.4 vs. 11.15 ng/mL, p=0.006; N=19 in May 2004 and N=8 August 2003) and after self-excavation (e.g., 34.1 vs. 14.04 ng/mL, p=0.025; N=18 in June–September 2004, N=9 in September 2003). Because all tortoises received the same dose of ACTH per gram of body weight, it appears that there may be either a seasonal change or a population difference in the adrenal gland's sensitivity to ACTH administration.

Long-term physiological responses to entombment

In an effort to determine potential long-term effects of burrow collapse, a subset of the 2003 (i.e., D12) and the 2004 (i.e., Oscars) study tortoises

whose burrows were collapsed were recaptured during the 2005 season (April–September). Their physiological parameters (e.g., CI, hematocrit, baseline corticosterone, and ACTH responsiveness) were compared with control groups of tortoises from their respective areas whose burrows had not been collapsed. Additionally, all of these parameters were compared between years for the experimental tortoises (i.e., those that had their burrows collapsed).

Condition index

An initial CI was calculated for animals collected in April-May 2005. There was no significant difference (p=0.12) in mean CI among animals from the collapse studies compared with each other or with control animals from the respective sectors (Figure 30). Difference in mean CI between sexes was not significant either within treatment groups or between the tortoises from the collapse studies and their controls.
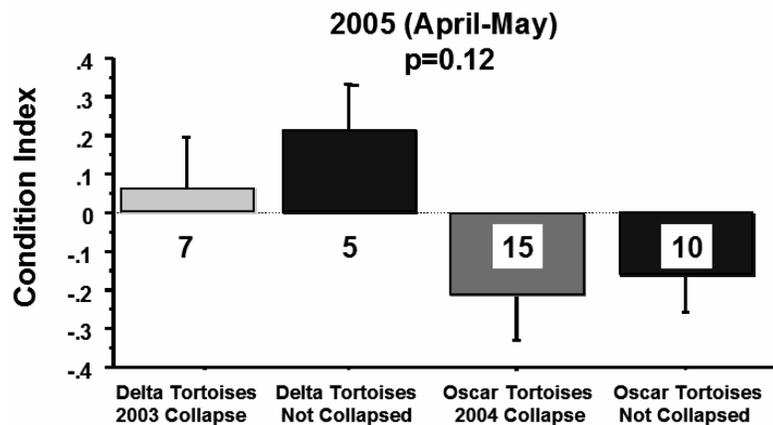
Figure 30. Mean CIs for tortoises captured in April-early May 2005. Groups consist of tortoises that were in burrow collapse studies in 2003 and 2004 and their control counterparts.

Change in mean CI was also compared between years in experimental animals. The CI value calculated when tortoises were first captured (i.e., before their burrows were collapsed) was compared to the CI value of the tortoise when it was first captured in 2005 (there was, thus, a 12-month time interval for the 2004 study tortoises and a 22-month interval for the 2003 study tortoises). No overall significant difference in mean CI was found between years for either collapse study (repeated measures ANOVA). However, a significant difference (p=0.04) was noted between sexes in the 2004 study, with females exhibiting a significant decrease in CI between years while males did not change (Figure 31b). The 2003 study

had a very small sample size but, again, it appeared the sexes were re-sponding differently between years. Unfortunately, control tortoises were not followed between years, so it is not known if these trends reflect a year effect or a response to burrow collapse.
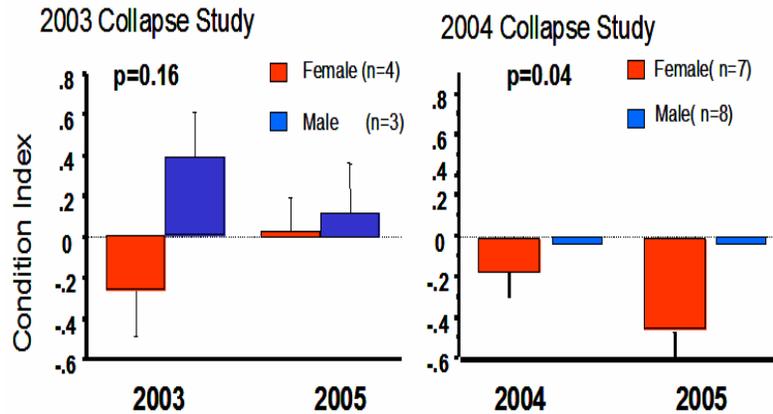
Figure 31. Mean CI for male and female tortoises whose burrows were collapsed in (a) 2003 and (b) 2004 and then recaptured in April-early May 2005.

Baseline corticosterone

During 2005, mean baseline corticosterone levels (taken upon removal of the tortoise from the trap) did not differ among experimental (those that had been in the previous collapse studies) and control tortoises when this measure was compared in April-early May, June, and September sample periods (Figure 32).

When baseline corticosterone levels of experimental animals were com-pared between years, it was found that baseline corticosterone at the first sample period in 2005 had decreased significantly (p=0.02) in 2004 males from the elevated levels they exhibited on the first sample after self-excavation. In the first 2005 sample, 2004 females did not exhibit this sig-nificant decrease because their post self-excavation levels were not ele-vated. This same trend was seen in the 2003 animals re-sampled in 2005: males exhibited a greater decrease than females, again because their post-excavation levels were higher than those of females at that time (the 2003 decrease was not significant due to the low sample size for that group re-captured in 2005).
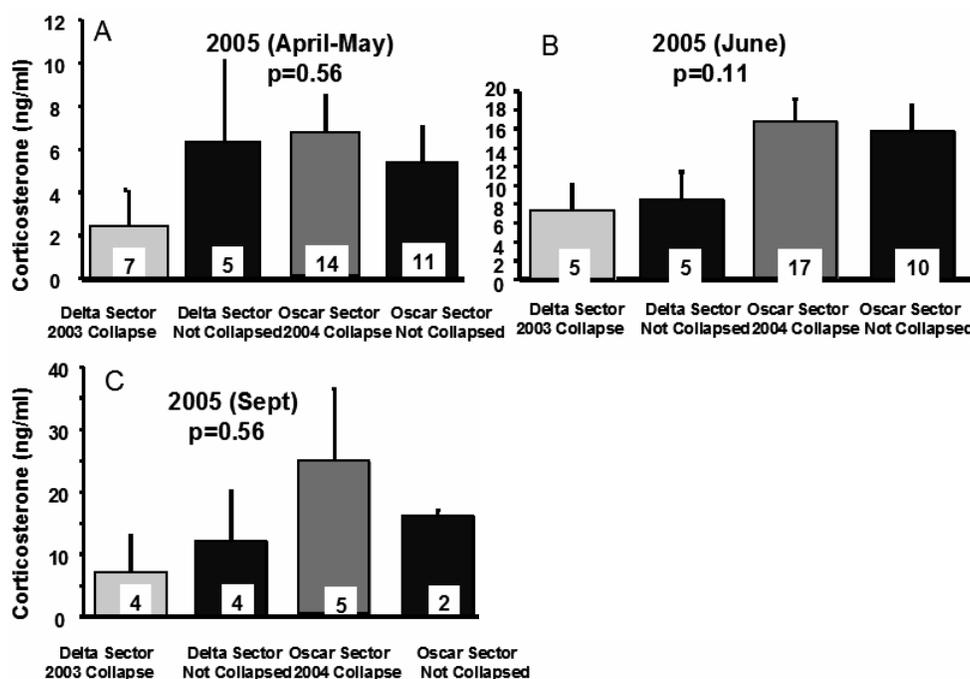
Figure 32. Mean baseline corticosterone levels for tortoises captured in 2005: (A) April-early May, (B) June, and (C) September. Groups consist of tortoises in previously burrow collapse studies in 2003/2004 and their 2005 control counterparts.

ACTH challenge

In the 2005 study, adrenal gland responsiveness was found to differ significantly between 2003 experimental (i.e., burrow collapse) tortoises and their control (i.e., previously undisturbed) counterparts. Tortoises whose burrows were collapsed in 2003 did not elevate their corticosterone levels significantly in response to ACTH when they were challenged in April 2005 (p=0.56) or when they were re-trapped and re-challenged in June 2005 (p=0.89), while the controls did elevate (p=0.02 and 0.05 respectively; Figure 33).

The 2003 and 2005 responses of experimental tortoises could not be statistically compared. In the 2003 study, tortoises either received ACTH or a saline injection. In 2005 only a few of the tortoises that were given ACTH in 2003 were recaptured, so the number of tortoises was not sufficient to do a repeated measures ANOVA comparison. However, the three tortoises that had been given ACTH in 2003 and 2005 exhibited similar nonresponses to the ACTH in both years.
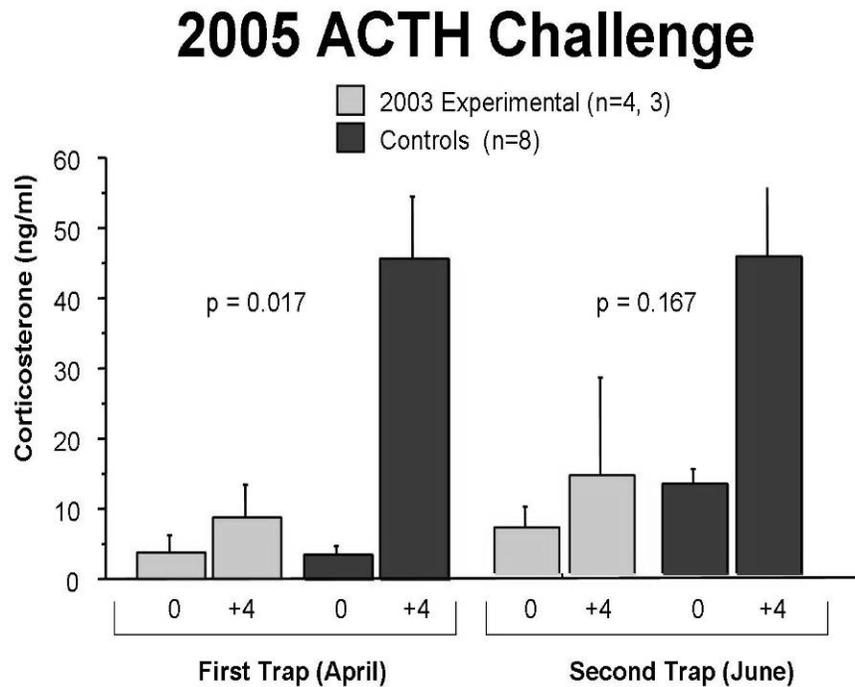
Figure 33. ACTH responsiveness, in April and June 2005, in tortoises whose burrows were collapsed in Fall 2003 vs. control tortoises.

When the 2004 experimental burrow collapse tortoises were recaptured and re-challenged in April 2005, their responsiveness to ACTH did not differ significantly from their control counterparts. Both groups had a significant increase in corticosterone in response to the ACTH challenge. In June 2005, however, the 2004 study tortoises did not respond significantly to the ACTH challenge, while the control tortoises did (p=0.05) (Figure 34). A repeated measures ANOVA analysis indicated that the experimental tortoises' June 2005 response differed from that observed in 2004 (i.e., the challenge after self-excavation) and in April 2005 (Figure 35).

2005 ACTH Challenge
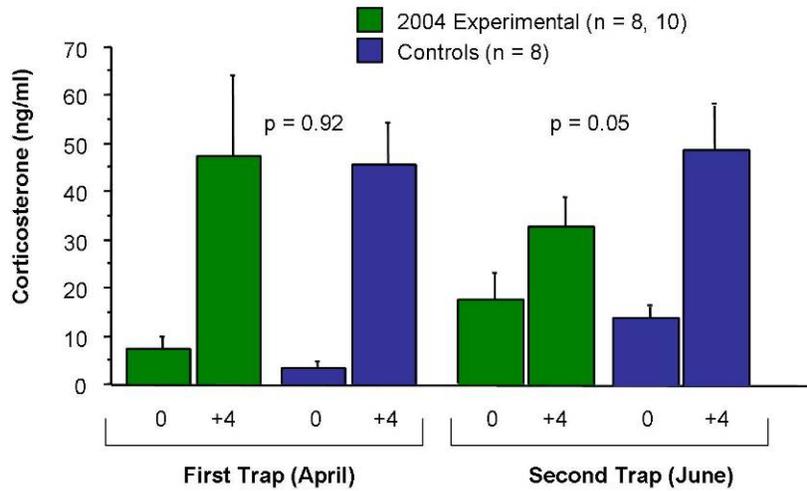
Figure 34. ACTH responsiveness, in April and June 2005, in tortoises whose burrows were collapsed in Summer 2004 vs. control tortoises.
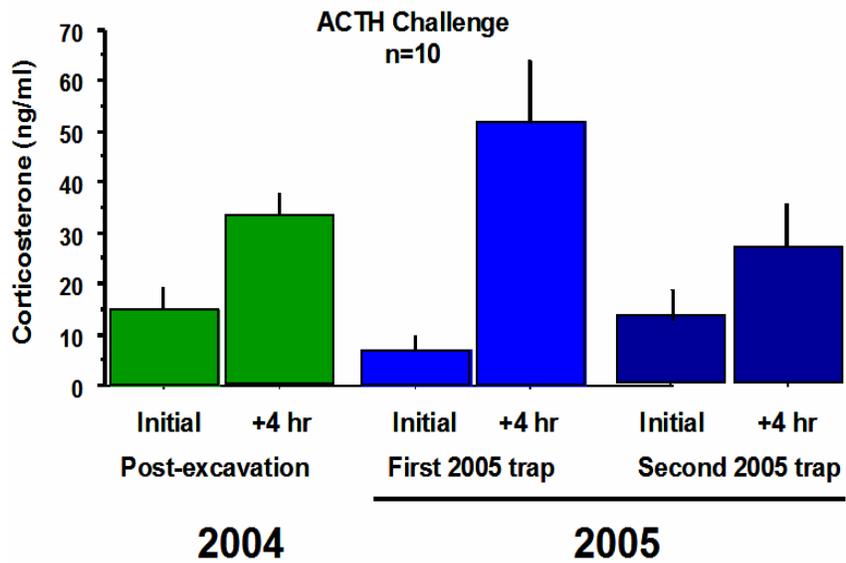
Figure 35. Relative ACTH responsiveness, in April and June 2005, in tortoises captured after self-excavation in 2004. June 2005 response not significant (p=0.39).

4 Conclusions

Auffenberg and Franz (1982) speculated that if gopher tortoise burrows were collapsed, entombed tortoises would die. In the three previously conducted burrow collapse studies (Landers and Buckner 1981; Diemer and Moler 1982; Wester 2004), there has been only one documented fatality (Wester 2004). In the study reported here, 41 out of 42 tortoises self-excavated. The tortoise that did not self-excavate was entombed 23 days from 7 to 30 September 2003. The tortoise was manually excavated to prevent it from entering the colder portion of the year while being entombed. At the time this decision was made, all of the 19 other tortoises in the 2003 study had self-excavated within 13 days of their burrow collapse date, and it appeared that the remaining tortoise might have been in difficulty. It may be that at this early stage of the study we were overly cautious in our concern for the tortoise's possible condition. However, given the results of the 2004 study in which 6 of 22 animals were entombed for longer than 23 days (up to 85 days) and the fact that the manually excavated tortoise showed normal levels of corticosterone, it appeared that it was not actually in distress. In retrospect, it would have been interesting to allow this animal to continue to be entombed to determine if it would have (1) self-excavated before the weather became colder, (2) remained in its burrow until spring and then self-excavated, or (3) died.

None of the tortoises were physically injured from the collapse events. There was, however, considerable variation in the time to self-excavation by the tortoises. Some tortoises self-excavated within hours of the collapse while one remained entombed for 85 days. First examined were physical and then physiological factors that might account for the variation in time to self-excavation among the tortoises.

First there was variation in the extent of the collapse among the burrows. In the first year of the study, two types of vehicles were used to collapse the burrows: an APC and a logging skidder. The skidder was more effective in collapsing the burrow than the APC, which had to repeatedly cross the burrow mouth to completely close it, and the APC did not seem to collapse the burrow along its length. The skidder could effectively cause more damage with a single pass. In the second year of the study, only a logging skidder was used and the actual length of burrow collapse it caused was measured. The extent of the collapse (determined by actual measurement) did

not, however, have a significant effect on the time it took tortoises to self-excavate. The only significant effect of the extent of collapse was on the site of self-excavation: the greater the length of the burrow collapse, the further from the burrow mouth was the exit point of the excavation. Importantly, there was also a significant ancillary effect of exit point on the subsequent abandonment status of the collapsed burrow. Burrows that were abandoned had a significantly greater average distance from self-excavation exit point to the original burrow mouth. Additionally, 46 and 41 percent (2003 and 2004, respectively) of the collapsed burrows were abandoned, frequencies that were approximately twice (i.e., 21 percent) that documented by Aresco and Guyer (1999b) for natural burrow abandonment in poor quality habitat.

Physical factors that did seem to affect the time until self-excavation were soil type and precipitation. Collapsed burrows were grouped into two categories—low clay and high clay content burrows—based on the SSURGO soil maps and a coarse-to-fine ratio analysis. Both of these data clearly separated the burrows into two groups. Difference in time until self-excavation was not significant in three of the four collapse groups (i.e., the two 2003 collapse dates and the first 2004 collapse date). Based on soil maps, 30 of 31 burrows in these first three collapse dates were found in soil that was low in clay content (i.e., 1–10 percent clay). The tortoises in the second 2004 collapse group (June 19) took significantly longer to self-excavate than the three previous groups. Six of 11 burrows that were in the second 2004 collapse group were in soils of high clay content (i.e., Esto 35–60 percent clay). When times to self-excavation were compared for tortoises whose burrows were in high clay versus low clay content, it was found that those from high clay content burrows were, again, significantly longer (Figure 36). Additionally, there was a large amount of precipitation just before the second collapse date of the 2004 study (Figure 19). Mean time to self-excavation associated with the low clay content burrows were not significantly different for similar burrows in 2003 and 2004 (Figure 36). Thus, it appears that the degree of clay in the soil impacts excavation time, while precipitation may have a secondary, modulating effect.

Kozlowski (1999) documented that some soils compact to a depth of more than 1 m under heavy traffic loads and that, when clay soils are wet, they compact more readily. This compaction would limit the porosity of the soil and might result in lower oxygen (O_2) and higher carbon dioxide (CO_2) levels within the burrow. Given these constraints, it would seem that tortoises would attempt to excavate sooner from these burrows. Instead, they

Soil Effect on Days Until Self Excavation
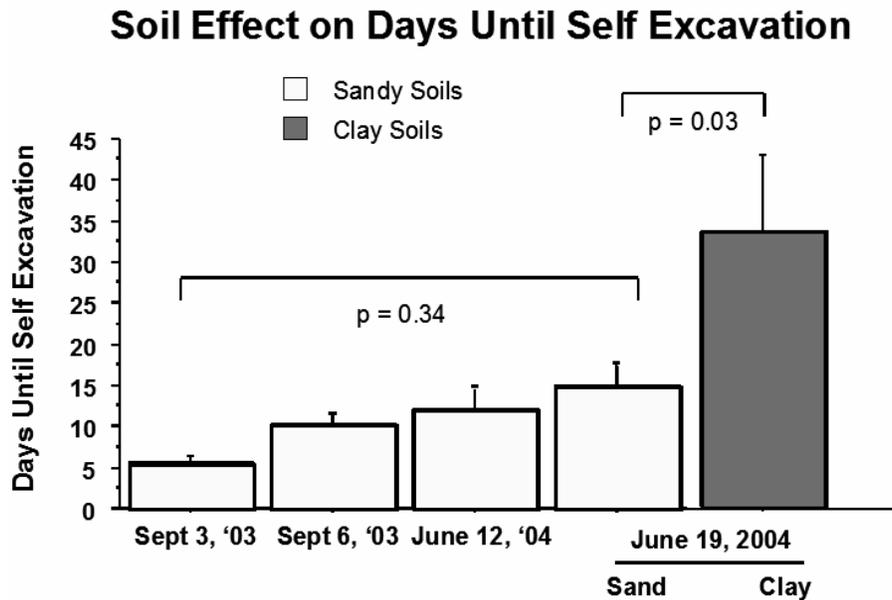
Figure 36. Effect of soil type on excavation times for each of the collapse dates in 2003 and 2004. Clay soils are defined as > 34 percent clay content and sandy soils are < 10 percent clay.

took significantly longer. It may be that heavily compacted clay is difficult to dig through, possibly accounting for the longer self-excavation times. Unfortunately, it could not be determined if the tortoises had been actively trying to self-excavate through compacted clay for some time or if they had remained quiescent in the burrow until some physiological or physical factor (hunger, thirst, excessive CO_2) caused them to emerge. Tortoises are known to be fairly tolerant of hypoxic and hypercarbic conditions (Ultsch and Anderson 1986) and may use the calcium carbonate found in their shells and bones as a buffer against lactic acid build-up (Jackson et al. 2000). Additionally, Means (1982) and Diemer (1992) both regularly observed tortoises below the water line in flooded burrows, again suggesting extreme tolerance to low O_2 levels. Thus, while tortoises may have been trying to emerge over some time from collapsed burrows in high clay content soil, it is also plausible to suggest they remained quietly in their burrow until they were triggered to emerge and then proceeded to do so rapidly. The trigger to emerge did not seem to be dehydration. No relationship existed between hematocrit (a crude indicator of dehydration) and the number of days until self-excavation or a change in hematocrit in pre- versus post-collapse samples.

Males and females did not differ significantly in the time it took them to self-excavate in either the 2003 or 2004 study. In 2003 females had slightly longer mean time to self-excavation than males (when the female that was manually excavated is removed from the analysis). The mean

time interval for 2003 females rises when the manually excavated female is included in the analysis, but this average is still not significantly different from that of males. In 2004 males tended to stay in the burrows longer than females but, again, no significant difference. Four of the last five tortoises in the 2004 study, which had extended entombment times, were males. Since the collapse dates of the 2004 study were 12 June and 19 June, it is likely that females had already laid their eggs for the season (Ott et al. 2000). Thus, the need to oviposit did not cause females to emerge in less time than males. However, females do start the vitellogenesis of the next year's clutch in late summer and fall (Rostal et al. 1994; Ott et al. 2000), and the energetic demands congruent with this process may have caused them to emerge earlier than males. On the other hand, males that remained entombed throughout the months of July-September were also undergoing a period of gonadal development (i.e., testicular growth and sperm maturation). In general, spermatogenesis is not considered as energetically costly a process as follicular development (Lagarde et al. 2003). So males could, perhaps, sustain this process by mobilizing fat stores. However, mating occurs during summer and fall in gopher tortoises and entombment during these months would cause males to miss mating opportunities, a loss that incurs a genetic cost.

Movement patterns

Collapse of gopher tortoise burrows and the subsequent entombment period did not significantly affect the tortoises' short-term movement behavior. The movements were not different pre- and post-collapse in either 2003 or 2004 and were consistent with what others have found (McRae et al. 1981; Diemer 1992; Eubanks et al. 2003). These studies all found that a large natural variance exists among tortoises in the number of times they move, the number of burrows they use, the mean distance moved, and home range sizes.

McRae et al. (1981) documented the mean number of burrows used for tortoises per month to be 1.5–3.0 for the months of May through September, with males being slightly higher in all months but May. Average moves per month were between 0.4–3.5, with males moving more frequently than females in all months between May and September (McRae et al. 1981). In both 2003 and 2004, males and females in this study exhibited similar patterns for burrow usage and the number of times moved before and after the collapse of their burrows. For example, in 2003 the mean number of burrows used and times moved before burrow collapse (August) were 3.3 and 3.1, respectively, while after burrow collapse (Sep-

tember) the means for each of these parameters were 2.0 and 1.7. In 2004 the pre-collapse (May–June) means were 2.1 burrows used and 1.6 times moved, while the post-collapse (June–September) means were 1.5 and 1.0, respectively.

In 2005 movements of tortoises that were subjects in the collapse studies in 2003 and 2004 did not differ when compared with the group of control tortoises at each of the two sites for number of burrows used, number of times moved, or HR. Tortoises in the 2003 study did not show any changes in movement when comparing their movements in 2005 and 2003. However, tortoises from the 2004 study used significantly more burrows (p = 0.03) and moved more times (p = 0.04) These results may have been caused by inter-year variance in environmental factors such as temperature and precipitation rather than burrow collapse. In both 2004 and 2005, the study was conducted from April to September and the amount of rainfall was drastically different (Table 3).

Table 3. The inter-year variation in precipitation and mean temperature for 2004 and 2005.

	April	May	June	July	August	September
2004 Precipitation (cm)	8.0	7.6	14.2	11.6	13.3	24.1
2005 Precipitation (cm)	18.1	12.2	22.4	23.8	16.5	1.3
2004 Mean Temperature (°C)	18.0	24.2	26.3	28.2	26.9	24.3
2005 Mean Temperature (°C)	17.3	21.6	26.3	28.1	27.9	26.9

Although tortoises did not exhibit significant changes in intra-year move-ment behavior or HR size after their burrows were collapsed, this result does not necessarily suggest that the burrow collapse and the subsequent entombment period do not pose a detriment to tortoises. Entombment caused by burrow collapse may interrupt the normal activity of tortoises, which may be expected to emerge every day or two for approximately 0.5 hour or more per day. In 2004 some tortoises were entombed for nearly 2 and 3 months (51 and 85 days, Figure 9). This entombment period would have limited their foraging opportunity at a time when they should be in-creasing their condition before over-wintering. It should be kept in mind, however, that a negative impact of prolonged entombment on the tor-toises' CI was not observed. In addition to losing the majority of a growing season, these particular tortoises may have also missed mating opportuni-ties, which occur primarily in the late summer and early fall.

Physiological responses

Condition index and hematocrit

A CI for the 2004 tortoises was calculated at (1) the beginning of the sea-
son (pre-collapse) and (2) when they were first captured after they self-
excavated (post-collapse). A repeated ANOVA found no significant change
in pre- versus post-collapse mean CI. Additionally, neither pre- nor post-
collapse CI correlated with the number of days until self-excavation. Since
the tortoises were kept from foraging for an extended period of time, it was
expected that the post-collapse CI would be lower than that calculated be-
fore the collapse. At the very least, it was expected that there would be
some correlation between the amount of time a tortoise was entombed and
what condition it was in either before or after the collapse. It may be that
the amount of time that the tortoises were entombed is within the normal
behavioral range for these animals to remain in their burrows and that this
inactive period does not cause sufficient weight loss to cause a change in
CI. In a telemetry study, Diemer (1992) observed a tortoise that did not
emerge from its burrow for 5 months. Additionally, a desert tortoise (*Go-
pherus agassizii*) that was pinned within its burrow by a fallen rock for 11
months, without food or water, lost only 4.2 percent of its body weight and
exhibited only minimal changes in standard blood chemistry measures
(Christopher 1999). So, even a 3-month entombment (the longest ob-
served) may not constitute a great challenge for these animals. However,
while gopher tortoises may naturally remain in burrows without eating for
an extended time, presumably tortoises in collapsed burrows are in more
hypoxic and water-deprived states than normally quiescent tortoises. Al-
ternatively, the lack of change in CI could have been due to a methodologi-
cal fault. A weight measurement of the tortoise was not taken just before
the collapse date but was instead taken early in the active season (just after
the winter period); it could be that tortoises gained weight in the interven-
ing time period. Since they were not measured at their maximal weight,
the predicted decrease in CI was not seen.

Another measure expected to reflect entombment time was hematocrit, a
crude indicator of dehydration. As for CI, a relationship between hema-
tocrit and days to self-excavation and mean levels were not found to be
significantly lower when animals were first captured after they emerged. It
may be that water vapor in the burrow, sustained by the porosity of the
soil, is sufficient to prevent dehydration. Alternatively, this result could
again be a methodological artifact. Tortoises were not always captured
immediately upon emergence. Although a trap was placed within a day of

emergence at the mouth of the burrow to which the animal relocated, there was a small time period in which the animal could have foraged or drank. This activity may have been enough to restore normal hematocrit levels. The range of variability for this parameter was very small, however, which would indicate every tortoise was able to rehydrate in a very short time. This occurrence seems unlikely.

In 2005 CIs of experimental tortoises (i.e., from the 2003 and 2004 burrow collapse studies) were compared with tortoises from the same respective areas that had not been handled at all in those years. No significant difference in mean CI was found among the groups, though the averages for the experimental tortoises tended to be lower than their unmanipulated counterparts. Also compared were the mean CIs of experimental tortoises between their collapse year and 2005. No significant between-year difference was found in the mean CIs of male and female tortoises from the 2003 study. However, males tended to have lower mean CIs, while females had mean higher CIs when they were recaptured in April 2005. Although the sexes exhibited a different response trend, the sample size was very small for a meaningful interpretation of this result. When the 2004 experimental tortoises were recaptured in 2005, it was found that females had significantly lower mean CIs than previously calculated, while that of males was unchanged. However, no correlation existed between CI and days until self-excavation for these females. Additionally, control females and males were exhibiting a similar CI pattern at this time of year. Thus, although the addition of a control group followed across years would have resulted in a more definitive comparison, it does not appear that being within a collapsed burrow for a period of time impacts long-term CI (at least as calculated in this study).

Baseline corticosterone

Baseline levels of corticosterone, a hormone associated with the stress response, did significantly increase the longer tortoises remained in their collapsed burrows in both the 2003 and 2004 burrow collapse studies. This significant, positive relationship was not an artifact of what the individual tortoises' baseline corticosterone levels were *before* the collapse: they had no relation to time to self-excavation or to their post-collapse baseline levels. It is important to note that the positive relationship was still evident in tortoises sampled days after they had self-excavated. Although there was variation in baseline corticosterone levels when the animals were trapped after they self-excavated (anywhere from 1 to 39 days), the factor that explained most of the variation in baseline corticosterone

levels was the number of days they were entombed. Thus, corticosterone levels appear to remain elevated for some time after self-excavation and may have, in fact, been much higher immediately after animals had emerged or even while they were entombed.

In the 2003 study (with shorter entombment times), the observed levels of corticosterone were almost all (15 of 17) within a range of values that, given studies of other populations at Fort Benning, would be considered normal (i.e., not stressed; Kahn et al. 2007). The data from the ACTH challenge experiment, however, indicate that the tortoises in the 2003 study may, in fact, have lower maximum levels of corticosterone than other populations. Thus, even these relatively low levels may indicate a stress response by these tortoises.

In the 2004 study (which had longer entombment times), half of the tortoises exhibited levels above 12 ng/mL; most of these elevated levels were observed in animals that had been entombed more than 10 days. Although these levels were elevated, most of them were not in the range of the absolute maximum values that could be elicited through an ACTH challenge. The majority of the elevated levels were, however, near the average maximum corticosterone response to ACTH in this population (i.e., approximately 30-40 ng/mL).

A transient increase in corticosterone levels may indicate an acute stressor, such as an encounter with a predator or researcher. However, elevated corticosterone is also important in mobilizing energy stores and promoting protein catabolism for gluconeogenesis (Wingfield et al. 1994, 1998) when an animal is experiencing low food availability. In a study on red-spotted garter snakes, males in relatively poor condition after emergence from hibernation exhibited significantly higher plasma corticosterone levels than males of higher condition sampled at that time (Moore et al. 2000). Elevated levels of corticosterone are also correlated with increased foraging behavior as well as seasonal migratory behavior (Emerson 2001; Lohmus et al. 2003). It may be that the observed positive relationship in corticosterone level with days until self-excavation relates to corticosterone's role in mobilizing energy stores to compensate for the lack of food intake. However, a significant decrease in CI was not seen in response to being entombed and the tortoise CI upon self-excavation did not correlate to their baseline corticosterone levels at that time. Additionally, there was no significant difference in mean CIs between the sexes, al-

though male and female tortoises exhibited very different corticosterone patterns in response to entombment time.

In both the 2003 and 2004 studies, a significant, positive relationship was found between baseline corticosterone of males at their first capture after self-excavation and days until emergence. Females did not exhibit this relationship. In 2004 the increased corticosterone levels seen in males in relation to entombment time could be because males tended to remain in the burrows longer. However, in 2003 when the excavation pattern did not differ between the sexes, males still exhibited this significant, positive relationship. Thus, males did seem to be responding differently to entombment than females. Again, the summer and fall months are the time that males would normally be increasing their movements to find females for mating. Although increased corticosterone levels can alter foraging behavior (Wingfield 1994) and has been implicated in increased locomotor behavior in general (Cash and Holberton 1999; Moore 2003), it has not been associated with increased movement behavior associated with finding mates. Additionally, a previous study on the annual sex steroid cycle of gopher tortoises found no seasonality in corticosterone levels in either females or males (Ott et al. 2000). So it is not likely that corticosterone is increasing to induce a seasonal change in male locomotory behavior (as it does in terms of bird migratory behavior). Instead, the increased levels do seem to be in response to the actual entombment times and may relate to potentially different levels of fat reserves in male versus female tortoises.

In the 2003 study, it was interesting to note that baseline corticosterone levels approximately 10 days following the first capture after self-excavation were significantly, positively correlated with excavation time in females, implying a delayed response to the collapse. However, this relationship is based on a small sample and driven by two points. No samples were taken at this time point in 2004 to confirm the response, however.

When mean baseline corticosterone levels were compared in 2005 experimental and control animals, from either the Delta or Oscar site, no significant difference was found between groups. Additionally, the experimental males, whose corticosterone levels were elevated when they were first captured after self-excavation, exhibited significantly lower levels of corticosterone when they were recaptured in April, June, and September 2005 (repeated ANOVA). Thus, it appears that, although a period of entombment within the collapsed burrows does elevate corticosterone

levels, especially in males, the effect for this parameter is transient (but see ACTH responsiveness below).

ACTH responsiveness

It was found that tortoises in the 2003 study did not exhibit a significant increase in corticosterone in response to an ACTH challenge after they self-excavated, though they had done so in the pre-collapse challenges. In contrast, the animals in the 2004 study did exhibit a significant increase in corticosterone in response to the challenge both before and after the collapse. Thus, at one site (Delta sector) at one time of year (September 2003), burrow collapse did seem to have an impact on the ability to significantly increase corticosterone if presented with a new challenge, whereas it did not in the other study (Oscar sector, June–September 2004). This difference between the two studies was not due to a difference in the ACTH itself, which was purchased from the same company and came from the same lot number. The lack of consistent impact of the collapse (especially in the study with longer entombment times) suggests that extended elevation of corticosterone levels (i.e., while animals were in the collapsed burrows and shortly after self-excavation) may not diminish the capacity of the adrenal gland to respond to a new challenge.

Maximal levels of corticosterone in response to the ACTH challenge were observed to be much lower in 2003 versus 2004 tortoises (for both the before and after burrow collapse samples). Because the same dose of ACTH was used in both studies, this difference in mean maximum levels of corticosterone was initially interpreted as a seasonal change in sensitivity to ACTH. It could be that, at this time of year, when a natural stressor initiates an ACTH surge, the adrenal gland will not respond as strongly as it could in another season. Given this possible seasonal component, the relatively low levels of corticosterone observed in 2003 tortoises may represent a stress response to the burrow collapse. Additionally, lack of a significant response to the post-collapse ACTH challenge may represent an important effect of burrow collapse on the animals, although a similar pattern was not seen in 2004.

This latter interpretation is further supported by the 2005 ACTH challenge results. In April and June 2005, when the tortoises from the Delta site whose burrows had been collapsed in September 2003 were re-challenged, these animals were again found to not significantly elevate their corticosterone, and their maximal levels were again significantly below those observed in tortoises from the Oscar site (both in 2004 and 2005) as well as

the control tortoises from the Delta site. These results indicate two things. First, the overall lower maximal levels of corticosterone found in the 2003 experimental tortoises represent neither a seasonal nor a site effect. For example, the same pattern was observed in September, April, and June, months that represent for the tortoises significantly different points in the annual cycle of food abundance, temperature and precipitation patterns, and reproduction. Additionally, control tortoises from the same site exhibited a significant response to the challenge, and their maximal levels were equivalent to those observed in tortoises from other sites. Second, if the responses were due to neither season nor site, then burrow collapse may have affected the short- and long-term ability of these tortoises to respond to an artificial challenge and, potentially, to new stressors in the environment. Specifically, tortoises exhibited significantly elevated levels of corticosterone in response to the challenge before the burrow collapse but not after the collapse, both in 2003 and in the April and June 2005 challenges approximately 18 months after the burrows were collapsed.

Of even more interest, the 2004 tortoises were able to mount a significant response to the challenge in April 2005 but not when re-challenged in June 2005. It could be that the repeated challenges (as well as the stress imposed by trapping and other manipulations) to the adrenal gland were exhausting its capacity to respond. Several factors argue against this idea, however. First, the control tortoises within this study year were also repeatedly challenged and did not show a diminished response. It is acknowledged, however, that these tortoises had not been trapped as many times as the 2003 and 2004 tortoises. Second, it seems unlikely that challenges/handling 8 months (the narrowest time period) earlier would have a continued effect over such a long time. If they did, one would expect to observe it in the April as well as the June sample. Third, repeated ACTH challenges have been done on tortoises in a relocation study at Fort Benning, and this diminishing of responsiveness to ACTH was not observed (Kahn 2006). This idea cannot be totally discounted, however, because no group that was handled a similar number of times without having their burrows collapsed has been studied.

In all cases, corticosterone did increase in response to the ACTH challenge but, in tortoises whose burrows had been collapsed (and, admittedly, repeatedly handled), it did increase significantly relative to their baseline levels. It could be that a period of entombment and prolonged elevated corticosterone levels can have long-term effects on adrenal responsiveness, but these effects are expressed only if tortoises come from a site or at

a time of year where they are being exposed to additional stressors (i.e., some sort of energy constraint).

5 Recommendations

It must be remembered that the original impetus for undertaking these studies came from an interest in the extent to which collapse of a burrow threatened the life of a tortoise (i.e., the degree to which "take" in the sense of the Endangered Species Act would result from a collapse). This information is relevant to management of tortoises in both the listed (i.e., Western Alabama, Mississippi, and Louisiana) population and the remainder of the Southeastern tortoise range. In this sense, it may be said as a result of these studies that "take" in this sense should be small under normal circumstances. Let us define for this purpose that "normal" means when a burrow is accidentally overrun during either forest management or military training activities. This excludes, however, actions which would repeatedly affect the same location, by numerous pieces of equipment over extended periods. Thus, the establishment of a skid trail or yarding area during forest harvest, or a bivouac or defilade site during a military training activity could certainly result in death of some tortoises.

Some potentially negative effects do result from burrow collapse, however. Depending on the time of year when it occurs, it may prevent a gravid female from depositing a clutch of eggs in a satisfactory location for them to hatch. At other times, mating opportunities could be decreased, an effect which, in some impacted habitats, could mean that the female did not mate at all in that year (Guyer et al. 2006). Commensal species, including listed and at-risk species that share burrows or use abandoned burrows, could be trapped in the collapsed burrow. The gopher frog (*Rana capito*) and indigo snake (*Drymarchon couperi*) come to mind, and would likely be unable to excavate as readily as a tortoise, and thus would be more likely to die. This means, then, that it is erroneous to suggest that no potential exists for negative effects among sensitive species, just that severe negative effects on tortoises are not likely.

Thus, it is believed that land and forest managers need not be overly concerned about keeping every piece of equipment out of areas known to contain tortoise burrows. Nor should it be absolutely necessary to mark every burrow location before entry, although this should have a beneficial effect if carried out and equipment operators know and understand that these are places to be avoided whenever possible. What must be avoided, how-

ever, is the establishment of intensive use activities, even temporary ones, on such sites.

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14. ABSTRACT

The gopher tortoise is a species of concern throughout the Southeast, and is a keystone species throughout its range. It is federally listed as "Threatened" in part of its range and has been proposed for listing elsewhere. During forest thinning and harvesting, and during military training exercises, tortoise burrows are often accidentally run over. No formal studies of the potential for tortoise injury resulting from burrow collapse had been conducted in the natural environment, however. This research was designed to determine the potential for tortoise injury from the direct crushing of the burrows or loss of life through inability to escape from a collapsed burrow. Forty intentional burrow collapses by heavy equipment were studied. Data were acquired on pre- and post-collapse movement patterns and several general health and physiological measures. No tortoises appeared to have any life-threatening injury, and all tortoises self-excavated, with excavation intervals ranging from an hour to 85 days. All tortoises remained within a normal home range of their collapsed burrow, resulting in little change in movement patterns after burrow collapse. Changes in home range, number of burrows used, daily movement patterns, or the mean distance moved by the tortoises does not appear significant.

15. SUBJECT TERMS

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